DR. ROMAN SLOUTSKY (Orcid ID : 0000-0002-0794-1255)

DR. MARGARET STRATTON (Orcid ID : 0000-0003-2686-9022)

Article type : Special Issue Review

FUNCTIONAL IMPLICATIONS OF CAMKII ALTERNATIVE SPLICING

Roman Sloutsky and Margaret M. Stratton

Department of Biochemistry and Molecular Biology University of Massachusetts, Amherst United States (mstratton@umass.edu)

Running title: CaMKII splice variants

Keywords: CaMKII, calcium-dependent kinase, splice variants, alternative splicing

Counts: 6325 words (including citations) and 5 figures.

Abstract

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is known to be a crucial regulator in the post-synapse during long-term potentiation. This important protein has been the subject of many studies centered on understanding memory at the molecular, cellular, and organismic level. CaMKII is encoded by four genes in humans, all of which undergo alternative splicing at the RNA level, leading to an enormous diversity of expressed proteins. Advances in sequencing technologies have facilitated the discovery of many new CaMKII transcripts. To date, newly discovered CaMKII transcripts have

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/EJN.14761

This article is protected by copyright. All rights reserved

Accepted Article

Accepted Article

been incorporated into an ambiguous naming scheme. Herein, we review the initial experiments leading to the discovery of CaMKII and its subsequent variants. We propose the adoption of a new, unambiguous naming scheme for CaMKII variants. Finally, we discuss biological implications for CaMKII splice variants.

Introduction

The topic of this review is the fascinating enzyme: Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). CaMKII is known to be essential to long-term potentiation (LTP), a key component of the cellular basis of memory (Herring and Nicoll, 2016). Clear evidence for this role derives from studying transgenic mice carrying a mutation in CaMKIIα at a key autophosphorylation site (Thr286Ala). These mutant mice displayed spatial learning deficits (Giese, Fedorov, Filipkowski, and Silva, 1998). Intriguingly, transgenic mice harboring mutations in CaMKIIα at other key autophosphorylation sites known to be inhibitory (Thr305Val/306Ala) exhibit a distinct memory phenotype where the mice displayed rigid learning (Elgersma, Fedorov, Ikonen, Choi, Elgersma, Carvalho, Giese, and Silva, 2002). The fact that the two genotypes produce distinct phenotypes suggests that CaMKII may indeed be playing multiple roles in memory formation and storage. These results, and others, have spurred decades of study on the role of CaMKII in memory.

In this review, we highlight the complexity of CaMKII at the sequence level. Several studies have detailed the implications of numerous splice variants (Sloutsky, Dziedzic, Dunn, Bates, Torres-Ocampo, Boopathy, Page, Weeks, Chao, and Stratton, 2020; Cook, Bourke, O'Leary, Zaegel, Lasda, Mize-Berge, Quillinan, Tucker, Coultrap, Herson, and Bayer, 2018; Suzuki, Suzuki, Yoshida, Kubo, Li, Okuda, Amanai, and Perry, 2010; Chang, Minahan, Merriman, and Jones, 2009; Tombes, Faison, and Turbeville, 2003), yet the nomenclature to distinguish one variant from another has long been a point of confusion in the field. We have compiled a timeline of the discovery of CaMKII and its specific splice variants over the past several decades and now propose a new naming scheme, which will allow for incorporation of any newly discovered variants. Finally, we comment on the biological ramifications of having this amazing diversity encoded into the primary sequence of CaMKII genes.

CaMKII gene architecture

This article is protected by copyright. All rights reserved

Accepted Article

CaMKII is encoded on four genes in vertebrates: CaMKIIα, β, γ, and δ. All four genes are expressed in the brain, but CaMKIIα and β are the predominant variants implicated in LTP (Incontro, Díaz-Alonso, Iafrati, Vieira, Asensio, Sohal, Roche, Bender, and Nicoll, 2018; Herring and Nicoll, 2016). CaMKIIγ is expressed throughout the body, including egg and sperm, where it plays a major role in fertilization (Yoon, Jellerette, Salicioni, Lee, Yoo, Coward, Parrington, Grow, Cibelli, Visconti, Mager, and Fissore, 2008; Escoffier, Lee, Yassine, Zouari, Martinez, Karaouzène, Coutton, Kherraf, Halouani, Triki, Nef, Thierry-Mieg, Savinov, Fissore, Ray, and Arnoult, 2016). CaMKIIδ is the major variant expressed in the heart and plays a role in cardiac pacemaking (Maier and Bers, 2002; Backs, Backs, Neef, Kreusser, Lehmann, Patrick, Grueter, Qi, Richardson, Hill, Katus, Bassel-Duby, Maier, and Olson, 2009). Each CaMKII subunit is comprised of a kinase domain (exons 1-10), regulatory segment (exons 11-12), variable linker region (exons 13-21), and a hub domain (exons 22-24) (Fig. 1A). Exon boundaries are highly conserved between all four genes across vertebrates (Fig. 1A, top). The kinase and hub domains are highly conserved across the four genes, with minimum 90% and 75% pairwise identity, respectively. The linker that connects the kinase and hub domains is highly variable in length and composition due to alternative splicing between linker exons. Additional splice sites are found within the kinase domain of CaMKIIδ (exon 6) and the hub domain of CaMKIIβ (exon 23) (Fig. 1B).

On close inspection of the splice sites at the codon level, it is clear that not all exon connections would be in the correct reading frame (Fig. 2A). For example, exon 12 always has a single ‘G’ and the 3’ end, so the subsequent exon must have 2 bases at the 5’ end to complete a codon. All four genes are conducive to having all linker exons spliced out, since exon 22 always contains 2 bases at the 5’ end. The requirement to remain in frame introduces a number of additional constraints on linker exon connectivity, which are summarized in Fig. 2B.

These complexities of CaMKII at the sequence level have led to some confusion over the years in terms of classifying variants in a consistent manner. We review here the history of CaMKII and its splice variants, as well as propose a naming scheme moving forward.

CaMKII splice variants

This article is protected by copyright. All rights reserved

Historical perspective

It was discovered in 1970 that the Ca^{2+}-dependent activation of cyclic 3',5'-nucleotide phosphodiesterase required a protein mediator (Cheung, 1971; Kakiuchi and Yamazaki, 1970; Cheung, 1970; Kakiuchi, Yamazaki, and Nakajima, 1970). Throughout the 1970s this protein was commonly referred to as a "calcium-dependent regulator" (CDR) (Schulman and Greengard, 1978b,a) of a much broader spectrum of Ca^{2+}-dependent biological processes (Cheung, 1980), now known as calmodulin. One such process mediated by calmodulin was found to be Ca^{2+}-dependent protein phosphorylation (Stull, Nunnally, and Michnoff, 1986; Edelman, Blumenthal, and Krebs, 1987). The late 1970s and early 1980s were filled with discoveries and characterization of protein kinases whose Ca^{2+}-dependent activation is mediated by calmodulin. Myosin light chain kinase (Dabrowska, Sherry, Aromatorio, and Hartshorne, 1978; Yagi, Yazawa, Kakiuchi, Ohshima, and Uenishi, 1978; Nairn and Perry, 1979) and phosphorylase kinase (Shenolikar, Cohen, Cohen, Nairn, and Perry, 1979; Cohen, Burchell, Foulkes, Cohen, Vanaman, and Nairin, 1978), which had already been known to be Ca^{2+}-dependent, were quickly shown to be Ca^{2+}/calmodulin-dependent (Ca^{2+}/CaM). Shortly thereafter, researchers discovered additional Ca^{2+}/CaM-dependent kinase activity in multiple tissues that was not associated with either myosin light chain kinase or phosphorylase kinase. In the brain, these were responsible for the Ca^{2+}-dependent phosphorylation of tryptophan 5-monooxygenase (Yamauchi and Fujisawa, 1980), tyrosine 3-monooxygenase (Yamauchi, Nakata, and Fujisawa, 1981), synaptic protein I (later known as synapsin I) (Kennedy and Greengard, 1981), tubulin (Goldenring, Gonzalez, McGuire, and DeLorenzo, 1983), and microtubule-associated protein 2 (Yamauchi and Fujisawa, 1982). A Ca^{2+}/CaM-dependent kinase acting on glycogen synthase was identified in liver (Payne and Soderling, 1980) and skeletal muscle (Woodgett, Tonks, and Cohen, 1982). Once purified, some of these Ca^{2+}/CaM-dependent kinases could also phosphorylate casein (Woodgett, Tonks, and Cohen, 1982; Fukunaga, Yamamoto, Matsui, Higashi, and Miyamoto, 1982), myelin basic protein, and histones (Goldenring, Gonzalez, McGuire, and DeLorenzo, 1983; Fukunaga, Yamamoto, Matsui, Higashi, and Miyamoto, 1982; Ahmad, DePaoli-Roach, and Roach, 1982). In each case, the molecular mass of the native enzyme was inferred to be in the range of 500 to 850 kDa, (Goldenring, Gonzalez, McGuire, and DeLorenzo, 1983; Woodgett, Tonks, and Cohen, 1982; Fukunaga, Yamamoto, Matsui, Higashi, and Miyamoto, 1982; Ahmad, DePaoli-Roach, and Roach, 1982), composed of subunits

This article is protected by copyright. All rights reserved

Accepted Article

ranging from 49 to 63 kDa (Yamauchi and Fujisawa, 1980; Goldenring, Gonzalez, McGuire, and DeLorenzo, 1983; Fukunaga, Yamamoto, Matsui, Higashi, and Miyamoto, 1982; Ahmad, DePaoli-Roach, and Roach, 1982; Payne, Schworer, and Soderling, 1983).

1983 was a particularly busy year in the investigation of Ca^{2+}/CaM-dependent kinases. Two Ca^{2+}/CaM-dependent kinases separable by fractionation had been found to phosphorylate distinct Ser/Thr sites on synapsin I (site I: CaMKI and site II: CaMKII) (Kennedy and Greengard, 1981). Site II kinase was partially (Kennedy, McGuinness, and Greengard, 1983), and then, crucially, fully purified (Bennett, Erondu, and Kennedy, 1983) from brain tissue. All three gel bands of the partially purified kinase were autophosphorylated (Kennedy, McGuinness, and Greengard, 1983). The complete purification provided evidence that the gel bands corresponding to 50, 58, and 60 kDa were indeed subunits of the kinase itself (Bennett, Erondu, and Kennedy, 1983). While it had already been shown that subunits of glycogen synthase kinase from both liver (Ahmad, DePaoli-Roach, and Roach, 1982) and skeletal muscle (Woodgett, Davison, and Cohen, 1983) autophosphorylate *in vitro*, the 50, 58, and 60/61 kDa gel bands of partially purified brain synapsin I site II kinase were shown to correspond to three major targets of Ca^{2+}/CaM-dependent phosphorylation previously identified in homogenates from brain and several other tissues (Schulman and Greengard, 1978b,a). Taken together, these results suggested for the first time that autophosphorylation is likely to be a physiologically relevant phenomenon (Kennedy, McGuinness, and Greengard, 1983; Bennett, Erondu, and Kennedy, 1983).

Also in 1983, Ca^{2+}/CaM-dependent glycogen synthase kinase (now known to be CaMKII) was purified from skeletal muscle as a 696 kDa holoenzyme consisting primarily of 58 kDa subunits with a minor contribution of 54 kDa subunits. A dodecameric holoenzyme made up of roughly nine 50 kDa and three 58/60 kDa subunits had been postulated a month earlier for synapsin I site II kinase based on a 650 kDa native holoenzyme (Bennett, Erondu, and Kennedy, 1983). For the purified glycogen synthase kinase, the authors hypothesized a quaternary structure of the dodecameric holoenzyme consisting of two stacked hexameric rings, based on the first-ever electron micrographs of native glycogen synthase kinase clearly showing a 6-fold symmetric hub-and-spokes arrangement, and on the exact 12-fold ratio of holoenzyme mass (696 kDa) to primary subunit mass (58 kDa)

This article is protected by copyright. All rights reserved

Accepted Article

Accepted Article

(Woodgett, Davison, and Cohen, 1983). Aside from their obvious structural similarities, little suggested that Ca^{2+}/CaM-dependent kinases from brain and glycogen synthase kinase from liver and skeletal muscle might be the same enzyme until one such kinase purified from brain was shown to effectively phosphorylate glycogen synthase purified from skeletal muscle (Iwasa, Fukunaga, Yamamoto, Tanaka, and Miyamoto, 1983). Then, based on extremely similar subunit compositions, phosphopeptide maps, and rates of phosphorylation across panels of substrates, first synapsin I kinase and glycogen synthase from skeletal muscle (McGuinness, Lai, Greengard, Woodgett, and Cohen, 1983a), and then another brain Ca^{2+}/CaM-dependent kinase (Woodgett, Cohen, Yamauchi, and Fujisawa, 1984), were all demonstrated indeed to be the same enzyme expressed in different tissues. This prompted synergy of separate investigations of synapsin I kinase (Kennedy and Greengard, 1981; Kennedy, McGuinness, and Greengard, 1983; Bennett, Erondu, and Kennedy, 1983), other brain Ca^{2+}/CaM-dependent kinases (Yamauchi and Fujisawa, 1980; Yamauchi, Nakata, and Fujisawa, 1981; Yamauchi and Fujisawa, 1982; Fukunaga, Yamamoto, Matsui, Higashi, and Miyamoto, 1982; Yamauchi and Fujisawa, 1983; Goldenring, Gonzalez, McGuire, and DeLorenzo, 1983; Iwasa, Fukunaga, Yamamoto, Tanaka, and Miyamoto, 1983) and glycogen synthase kinase from multiple tissues (Payne and Soderling, 1980; Woodgett, Tonks, and Cohen, 1982; Ahmad, DePaoli-Roach, and Roach, 1982; Woodgett, Davison, and Cohen, 1983; Payne, Schworer, and Soderling, 1983). The authors declared the enzyme "a multifunctional calmodulin-dependent protein kinase that mediates many of the actions of Ca^{2+} in various tissues" (McGuinness, Lai, Greengard, Woodgett, and Cohen, 1983a).

In late 1983, the name Ca^{2+}/CaM-dependent kinase II (CaMKII) appeared for the first time in conference abstracts (Lai, McGuinness, and Greengard, 1983; McGuinness, Kelly, Ouimet, and Greengard, 1983b). The origin of the name is not explicitly clear from the literature. In 1980, Yamauchi and Fujisawa (Yamauchi and Fujisawa, 1980) identified three distinct brain Ca^{2+}/CaM-dependent kinases eluting in separate gel filtration fractions, with a fourth eluting in the void volume, and referred to these as kinases I through IV. Their analysis suggested that kinase I corresponded to phosphorylase kinase, kinase III corresponded to myosin light chain kinase, kinase IV was likely a heterogeneous mixture of aggregates, while kinase II was an unknown kinase. Starting in 1982, Yamauchi and Fujisawa refer to this enzyme as CaM-dependent protein kinase (kinase II) (Yamauchi

This article is protected by copyright. All rights reserved

and Fujisawa, 1982, 1983). Separately, in 1981 Kennedy and Greengard had observed two distinct brain Ca^{2+}/CaM-dependent kinases (Kennedy and Greengard, 1981), each phosphorylating synapsin I at a unique site. Titles of 1983 conference abstracts from the Greengard lab referred to CaMKII (Lai, McGuinness, and Greengard, 1983; McGuinness, Kelly, Ouimet, and Greengard, 1983b), while another abstract from the same lab referred to CaMKI (Nairn and Greengard, 1983). A 1984 conference abstract by Nairn and Greengard (Nairn and Greengard, 1984) described CaMKI as distinct from CaMKII. Nestler and Greengard stated that CaMKI phosphorylates site 1 on synapsin I, while CaMKII phosphorylates sites 2 and 3, citing the conference abstracts from 1983 (Nestler and Greengard, 1984). So, although the first research paper on CaMKI did not appear until 1987 (Nairn and Greengard, 1987), it appears that CaMKI and CaMKII may have been named at the same time in 1983, based on the sites they phosphorylate on synapsin I. Furthermore, CaMKIII appears to have been identified as distinct from CaMKI and CaMKII and named by Nairn, Bhagat, and Palfrey around the same time (Palfrey, 1983; Nairn, Bhagat, and Palfrey, 1985). However, this naming scheme was not universally adopted until later, as in 1986 "type II Ca^{2+}/calmodulin-dependent protein kinase" (Miller and Kennedy, 1986), and even simply "multifunctional calmodulin-dependent protein kinases" (Stull, Nunnally, and Michnoff, 1986) were also used.

Most critical to the topic of this review, in 1983 Mary Kennedy's lab named the 50 kDa, 60 kDa, and 58 kDa subunits of "calmodulin-dependent synapsin I kinase" as α, β, and β', (Bennett, Erondu, and Kennedy, 1983). Subunits distinguishable by SDS-PAGE analysis had previously been named α and β (Ahmad, DePaoli-Roach, and Roach, 1982), and ρ and σ (Goldenring, Gonzalez, McGuire, and DeLorenzo, 1983), but these names did not persist beyond the publications in which they were introduced. Bennett *et al.* correctly distinguished α from β/β' based on similar, but not identical phosphopeptide maps of their phosphorylated forms and on selective binding by a monoclonal antibody. Once substantial evidence had accumulated for "isozymes" comprised of multiple polypeptide subunits being present in multiple tissues (Kelly, McGuinness, and Greengard, 1984; Schworer, McClure, and Soderling, 1985; Miller and Kennedy, 1985; Shenolikar, Lickteig, Hardie, Soderling, Hanley, and Kelly, 1986), the stage was set for a definitive determination of subunit expression by nucleotide sequencing.

This article is protected by copyright. All rights reserved

The first two coding sequences of brain CaMKII were reported almost simultaneously by three groups in 1987. cDNA encoding the brain polypeptide designated α (Bennett, Erondu, and Kennedy, 1983) was identified from brain cDNA libraries by hybridization with synthetic oligonucleotides designed to match tryptic fragments derived from the 50 kDa brain CaMKII subunit and sequenced by Edman degradation (Lin, Kapiloff, Durgerian, Tatemoto, Russo, Hanson, Schulman, and Rosenfeld, 1987). Another group identified the cDNA encoding the β polypeptide by screening proteins expressed from a brain cDNA expression library (Bennett and Kennedy, 1987) against anti-CaMKII antibodies they had previously raised (Miller and Kennedy, 1985). A third group used a similar antibody screening strategy to identify and sequence an α fragment cDNA (Hanley, Means, Ono, Kemp, Burgin, Waxham, and Kelly, 1987). The α and β full length cDNAs were sequenced to derive predicted 478 amino acid α (Lin, Kapiloff, Durgerian, Tatemoto, Russo, Hanson, Schulman, and Rosenfeld, 1987) and 542 amino acid β (Bennett and Kennedy, 1987) polypeptides with molecular weights of 54 kDa and 60 kDa, respectively. Homology analysis of both proteins indicated N-terminal kinase domains similar in sequence to previously determined Ca^{2+}/CaM-dependent phosphorylase and myosin light chain kinases, and several other protein kinases (Bennett and Kennedy, 1987; Hanley, Means, Ono, Kemp, Burgin, Waxham, and Kelly, 1987; Lin, Kapiloff, Durgerian, Tatemoto, Russo, Hanson, Schulman, and Rosenfeld, 1987). The kinase domains were followed by stretches of basic residues similar to calmodulin binding sites previously identified in myosin light chain and phosphorylase kinases (Blumenthal, Takio, Edelman, Charbonneau, Titani, Walsh, and Krebs, 1985; Lukas, Burgess, Prendergast, Lau, and Watterson, 1986). A synthetic 25 amino acid peptide, derived from the putative CaMKIIα calmodulin binding site and flanking sequence, bound to calmodulin in the presence of Ca^{2+}. The remaining C-terminal amino acid sequences of both α and β lacked any known homology, but were correctly hypothesized to be involved in oligomerization (now known as the hub domain) (Lin, Kapiloff, Durgerian, Tatemoto, Russo, Hanson, Schulman, and Rosenfeld, 1987). Two of seven β cDNA clones had identical in-frame 45 nucleotide deletions relative to the remaining five clones, encoding a 58 kDa predicted polypeptide missing 15 residues relative to the 60 kDa β polypeptide. Based on this the authors correctly hypothesized that the 58 kDa gel band they had previously designated β′ (Bennett, Erondu, and Kennedy, 1983) reflected a distinct polypeptide species resulting from alternative splicing of the β transcript, rather than a product of proteolysis (Bennett and

This article is protected by copyright. All rights reserved

Accepted Article

Kennedy, 1987). We now know this 45 nucleotide sequence to be CaMKIIβ exon 17 (Fig. 4). Comparison of α and β/β′ coding sequences suggested that the two were products of different, but homologous genes (Hanley, Means, Ono, Kemp, Burgin, Waxham, and Kelly, 1987; Lin, Kapiloff, Durgerian, Tatemoto, Russo, Hanson, Schulman, and Rosenfeld, 1987). In a 1988 follow-up to the earliest 1987 report (Bennett and Kennedy, 1987), cDNAs for all three polypeptides were identified from the same brain cDNA libraries, confirming the distinct genomic origins of α and β/β' and reinforcing the case for the alternative splicing origin of β and β′ (Bulleit, Bennett, Molloy, Hurley, and Kennedy, 1988).

Over the following two years, transcripts from two additional CaMKII genes were identified and the corresponding polypeptides named γ and δ, in keeping with the existing naming scheme (Tobimatsu, Kameshita, and Fujisawa, 1988; Tobimatsu and Fujisawa, 1989). In 1988, a brain cDNA library was screened for CaMKII coding sequences by hybridization to an oligonucleotide complementary to a fragment of the previously published CaMKIIβ coding sequence (Bennett and Kennedy, 1987). In addition to four clones matching the published α coding sequence (Lin, Kapiloff, Durgerian, Tatemoto, Russo, Hanson, Schulman, and Rosenfeld, 1987), four clones matched β (Bennett and Kennedy, 1987), and an additional clone was found to encode a highly homologous, but distinct 527 amino acid, 59 kDa polypeptide, designated γ. Based on the alignment of α, β, and γ, the authors noted two regions of greater homology at the N-terminal (approximately 315 residues) and C-terminal (approximately 150 residues in γ) ends of all three sequences, flanking a shorter middle region (approximately 60 residues in γ) with significantly lower homology, which the authors designated the divergent region ("D-region"), and suggested may not be "essential for kinase activity" (Tobimatsu, Kameshita, and Fujisawa, 1988). We now know the homology among linker exons to be substantially higher than initially appreciated (Fig. 2A), and the perceived divergence to have resulted from alternative splicing of linker exons. The following year, the same group used this β antisense oligonucleotide screening approach to isolate CaMKII cDNAs from a cerebellum cDNA library. In addition to α, β, and the γ they had already identified, they sequenced a fourth distinct cDNA sequence encoding a 533 amino acid, 60 kDa polypeptide, which they designated δ. By the authors′ count, this brought the number of known CaMKII coding sequences to five: α, β, β′, γ, and δ (Tobimatsu and Fujisawa, 1989). Although the authors presumed that "[f]urther investigation [would]

This article is protected by copyright. All rights reserved

reveal the existence of additional members of the CaM-kinase II gene family", they had already identified the fourth and final CaMKII-encoding gene in mammals. With all four genes now identified, detection of additional splice variants became substantially easier by using targeted PCR with primers designed against the known coding sequences. Now, the stage was set for an explosion in the number of identified variants. With any explosion, there is an associated level of chaos. Since it could not be known in advance how many variants would be discovered, initial CaMKII variant naming strategies differed from group to group. This was compounded by the fact that many papers were published simultaneously, not allowing enough of an incubation time to adopt a uniform naming scheme in the field.

Nearly all preceding work had been carried out in rat and rabbit tissues. In 1991, a second α splice variant containing 33 additional nucleotides, now known as linker exon 15, was identified in monkey visual cortex and designated α-33 (Benson, Isackson, Gall, and Jones, 1991). When the same variant was detected in rat brain four years later, it was designated α_B, presumably because of the different species (Brocke, Srinivasan, and Schulman, 1995). Identification of γ and δ variants by multiple groups occurred so rapidly in 1993 and 1994 that near-simultaneous publications repeatedly assigned different names to the same δ variants. One manuscript identified three new δ splice variants in addition to the original δ (renamed as δ_1) and labeled the new variants δ_2-δ_4 (Schworer, Rothblum, Thekkumkara, and Singer, 1993). While that manuscript was in review, another manuscript identified a different collection of five new δ variants and named these δ_2-δ_6, with the original variant again renamed δ_1 (Mayer, Möhlig, Schatz, and Pfeiffer, 1993). However, δ_4 in the first publication corresponded to either δ_5 or δ_6 in the second publication, due to ambiguity at the C-terminal of CaMKIIδ transcripts, which was not sequenced in the first manuscript. A third manuscript defined two new variants: δ_B (= δ_3) and δ_C (= δ_2) (Edman and Schulman, 1994). A fourth manuscript submitted at the same time labeled the corresponding variants δ-c, δ-b, and δ-a (Zhou and Ikebe, 1994). Authors of the second manuscript (Pfeiffer and colleagues) adopted the numbering scheme laid out in the first manuscript in their subsequent publications (Mayer, Möhlig, Schatz, and Pfeiffer, 1994a). Nonetheless, the conflicting number and letter naming schemes persisted. Similarly, in human T lymphocytes, the original γ was renamed γ_A, and additional variants were named γ_B and γ_C

This article is protected by copyright. All rights reserved

Accepted Article

Accepted Article

(Nghiem, Saati, Martens, Gardner, and Schulman, 1993). The fourth manuscript acknowledged this naming scheme, but retained their own γ-a, γ-b, and γ-c designations for the corresponding variants detected in rat aorta smooth muscle, presumably because the sequence of γ exon 16 differs between rat and human (Zhou and Ikebe, 1994). These variants and their given names are summarized in Fig. 5.

Numerous additional variants of γ and δ were identified between 1995 and 2003, with conflicting naming schemes and occasional name clashes (Kwiatkowski and McGill, 1995; Mayer, Möhlig, Idlibe, and Pfeiffer, 1995; Tombes and Krystal, 1997; Singer, Benscoter, and Schworer, 1997; Takeuchi and Fujisawa, 1998; Hoch, Meyer, Hetzer, Krause, and Karczewski, 1999; Takeuchi, Yamamoto, Matsumoto, Kimura, Katsuragi, Miyakawa, and Miyamoto, 1999; Kwiatkowski and McGill, 2000; Takeuchi, Yamamoto, Fukunaga, Miyakawa, and Miyamoto, 2000; Gangopadhyay, Barber, Gallant, Grabarek, Smith, and Morgan, 2003) (Fig. 5). During the same period, a number of β variants were also discovered (Urquidi and Ashcroft, 1995; Brocke, Srinivasan, and Schulman, 1995; Tombes and Krystal, 1997; Bayer, Harbers, and Schulman, 1998; Bayer, Löhler, Schulman, and Harbers, 1999; Wang, Wu, Zhou, Sun, and Pei, 2000) and, fortunately, name clashes were avoided (Fig. 5). CaMKIIβ was also the first CaMKII gene to have its genomic location identified in mouse through a fortuitous accident in 1992 (Karls, Müller, Gilbert, Copeland, Jenkins, and Harbers, 1992). The genomic location of the CaMKIIα was determined over the course of multiple targeted studies in 1995 and 1996 (Olson, Massé, Suzuki, Chen, Alam, and Kelly, 1995; Nishioka, Shiojiri, Kadota, Morinaga, Kuwahara, Arakawa, Yamamoto, and Yamauchi, 1996). Genomic locations of CaMKIIγ and δ would not be known until the completion of human genome, reported in a 2003 review of CaMKII alternative splicing by Tombes and colleagues (Tombes, Faison, and Turbeville, 2003). 2003 also marked the effective end of the CaMKII splice variant discovery explosion, with the identification of several rare γ variants in smooth muscle (Gangopadhyay, Barber, Gallant, Grabarek, Smith, and Morgan, 2003). Variants labeled γ1-γ3 were reported in egg cells in 2009 (Chang, Minahan, Merriman, and Jones, 2009), but these matched variants reported earlier in other tissues (Zhou and Ikebe, 1994; Nghiem, Saati, Martens, Gardner, and Schulman, 1993; Takeuchi and Fujisawa, 1998) (Fig. 5). The intriguing CaMKIIβ variant where a splicing event causes a shorter hub version was first reported as β6 in 2000 (Wang, Wu, Zhou, Sun, and Pei, 2000). Additional short hub

This article is protected by copyright. All rights reserved

Accepted Article

variants were recently discovered and named βH and βeH in 2018 (Cook, Bourke, O'Leary, Zaegel, Lasda, Mize-Berge, Quillinan, Tucker, Coultrap, Herson, and Bayer, 2018), although the full coding sequences of these variants were not reported. The most crucial findings from the past 40 years of CaMKII splice variant discovery are highlighted in the timeline in Fig. 3.

With the limits of less-abundant variant discovery by traditional PCR and Sanger sequencing approaches apparently reached, no additional variants were identified between 2003 and present day, until a recent study used an Illumina sequencing strategy to detect an unprecedented number of variants of all four genes, including numerous previously un-described variants, in three human hippocampus samples (Sloutsky, Dziedzic, Dunn, Bates, Torres-Ocampo, Boopathy, Page, Weeks, Chao, and Stratton, 2020). Across the four sequenced samples, 3 CaMKIIα, 14 CaMKIIβ, 11 CaMKIIγ, and 6 CaMKIIδ transcripts were robustly detected, with nearly 80 variants detected in at least one sample. A brief summary of the highest-detected variants is presented in the next section. Detection of this unexpectedly large number of variants from individual tissue samples is nearly impossible by traditional sequencing approaches because of the three to four orders of magnitude differences in variant detection level and the extreme difficulty of resolving the variants as individual bands on agarose DNA gels.

Systematic splice variant nomenclature

We propose the implementation of a new naming scheme for CaMKII splice variants using a simple formula for composing variant names: 1) species, 2) gene identity, 3) incorporated exons. For example, human CaMKIIα(14,15,18) would designate the variant of CaMKIIα where all linker exons (14, 15, and 18) are incorporated. For variants that contain zero linker exons, we propose designating these with -0, where CaMKIIα with no linker exons would be: CaMKIIα-0. There are additional splice sites within exons of certain genes: exon 14 in CaMKIIβ and δ, exons 16, 18, and 19 in CaMKIIγ, and exon 23 in the hub domain of CaMKIIβ. Splicing at one of these sites produces a shortened version of the corresponding exon. In these cases, the full version of the exon should be designated 'a', while the short version should be designated 'b' to indicate truncation at the internal splice site. For example, human CaMKIIβ(13, 14b,16,18,23a) designates a variant of CaMKIIβ where exon 14 is spliced to produce the shorter form, while exon 23 is not spliced at its secondary site,

This article is protected by copyright. All rights reserved

producing its full length form. Finally, CaMKIIδ encodes two versions of exon 6 that differ at 5 of the 24 residues encoded by that exon. This is different from internal splice sites, as both exons are of equal length and are encoded in the genome sequentially with an intron between them. For these, we propose using 6v1 and 6v2 nomenclature to designate the more-frequently (6v1) and less-frequently (6v2) incorporated versions of the exon. CaMKIIδ variants should always incorporate 6v1 or 6v2 in their names. This is a flexible way to efficiently catalog currently documented CaMKII variants. Importantly, this strategy allows incorporation of newly discovered variants with any exon connectivity.

As examples of the proposed nomenclature, here we will summarize the highest-detected human hippocampal variants in (Sloutsky, Dziedzic, Dunn, Bates, Torres-Ocampo, Boopathy, Page, Weeks, Chao, and Stratton, 2020). CaMKIIα(14,18) (30-residue linker) and CaMKIIβ(13,14b,16,17,18) (93-residue linker) were the highest-detected splice variants of the corresponding genes, accounting for 70-95% of α reads and 36-56% of β reads, respectively. There were no consensus top variants of CaMKIIγ or CaMKIIδ. However, CaMKIIγ(14,16a,17,18b) (69-residue linker) averaged the most reads across all samples, ranging from 24-38%, and CaMKIIδ(6v1,14b,18) (28-residue linker) accounted for 26-53% of reads. A version of the CaMKIIδ variant incorporating exon 6v2, CaMKIIδ(6v2,14b,18) (28-residue linker), was also detected in all samples.

Biological impact of CaMKII alternative splicing

Protein-protein interactions and sub-cellular localization facilitated by linker exons

There are many facets to the biological impact of the variable linker in CaMKII. Given the high degree of conservation between the four vertebrate CaMKII genes, the linker is the most obvious candidate for the region that confers unique attributes to each splice variant. One way nature has exploited this is to encode binding sites for specific interaction partners in individual exons. This facilitates a diverse set of interacting partners for each splice variant. To date, clear roles have only been identified for two exons. One is F-actin binding, a functionality encoded on exon 13 (encoded only in CaMKIIβ and γ genes) (O'Leary, Lasda, and Bayer, 2006; Khan, Downing, and Molloy, 2019). Thus, CaMKIIβ and γ are able to localize to F-actin as long as exon 13 is spliced in. Exon 15,

This article is protected by copyright. All rights reserved

Accepted Article

encoded in all genes except CaMKIIβ, contains a nuclear localization sequence, facilitating translocation from the cytoplasm to the nucleus.

There are likely other binding sites encoded in, or facilitated by, the linker that have yet to be discovered. Interactions that have been shown to be variant- or gene-specific may indicate involvement of the variable linker region. Indeed, Tsien and colleagues worked out a complex mechanism whereby specific variants of CaMKII (α, β, γ) are recruited to the L-type Ca^{2+} channel (LTCC) or NMDA receptor in an activation-dependent manner (Ma, Groth, Cohen, Emery, Li, Hoedt, Zhang, Neubert, and Tsien, 2014). CaMKIIγ contains a nuclear localization sequence (NLS, exon 15), causing it to translocate to the nucleus with Ca^{2+}/CaM upon phosphorylation by CaMKIIα/β. This signaling cascade ultimately results in gene expression changes that drive synaptic plasticity and long-term memory (Ma, Groth, Cohen, Emery, Li, Hoedt, Zhang, Neubert, and Tsien, 2014). CaMKIIβ does not encode exon 15, and the CaMKIIα splice variant containing exon 15 is not strongly expressed in the hippocampus (Sloutsky, Dziedzic, Dunn, Bates, Torres-Ocampo, Boopathy, Page, Weeks, Chao, and Stratton, 2020). Thus, CaMKIIα/β is incapable of translocating to the nucleus.

In an elegant study from Colbran and colleagues, a binding site on the N-terminal domain of LTCC as well as residues on CaMKII required for binding that site were elucidated (Wang, Marks, Perfitt, Nakagawa, Lee, Jacobson, and Colbran, 2017). The required residues are localized on the kinase domain, and are present in all variants – yet only 2-3 variants of CaMKII (α/β, γ) are recruited to the LTCC. It is feasible that the variable linker may also interact with the kinase domain to facilitate or block specific interactions, but further studies are needed to fully understand all of the interactions between CaMKII and the CaMKII anchoring proteins (Colbran, 2004) / CaMKII-Associated Proteins (Robison, Bass, Jiao, MacMillan, Carmody, Bartlett, and Colbran, 2005) (CaMKAPs). Colbran and colleagues also performed a comprehensive mass spectrometry analysis of phosphorylation sites on CaMKII as well as CaMKAP identification (Baucum, Shonesy, Rose, and Colbran, 2015). Extensive phosphorylation is a characteristic of disordered protein regions (Gao and Xu, 2012), and many phosphorylation sites in the variable linkers of CaMKIIα and β were identified in this study, including sites within the actin-binding domain of CaMKIIβ. There are additional annotated and predicted phosphorylation sites within the linker region for all four CaMKII genes, and especially for longer

This article is protected by copyright. All rights reserved

linker variants (CaMKIIβ, γ, δ) (Jehl, Manguy, Shields, Higgins, and Davey, 2016). Nuclear translocation enabled by the NLS in exon 15 is conditional on the phosphorylation state of adjacent serine residues, also contained in exon 15 (Brocke, Srinivasan, and Schulman, 1995; Heist, Srinivasan, and Schulman, 1998; Ma, Groth, Cohen, Emery, Li, Hoedt, Zhang, Neubert, and Tsien, 2014; Ma, Li, and Tsien, 2015). The roles of many other linker phosphorylation sites remain unknown. Additionally, the linker contains sites predicted to be acetylated and ubiquitylated that have yet to be explored. Post-translational modifications within the linker region may facilitate structural changes, which in turn would have significant functional ramifications for intrinsic activity, interactions with other proteins, or changes in protein lifetime (Bah and Forman-Kay, 2016).

Structural implications of alternative splicing

There are two intriguing alternative splice sites outside the variable linker region. A splice site in exon 23 of CaMKIIβ can produce a short version of the hub domain with 26 residues removed (Wang, Wu, Zhou, Sun, and Pei, 2000). Deletion of these residues would require a significant structural rearrangement to maintain the fold of the hub domain (Fig. 1B). A recent study employed this short hub variant in cellular assays and showed that, impressively, it does appear to assemble into a mixed oligomer when expressed with wild-type CaMKIIβ (Cook, Bourke, O'Leary, Zaegel, Lasda, Mize-Berge, Quillinan, Tucker, Coultrap, Herson, and Bayer, 2018). The other unique splice site is at exon 6 of the CaMKIIδ kinase domain. The CaMKIIδ gene encodes two versions of this exon, with one or the other, but never both, spliced into CaMKIIδ transcripts. The two resulting versions of CaMKIIδ kinase domain have not been studied to date, nor, to our knowledge, has this alternative splice cite been reported previously. The only record of the two versions of CaMKIIδ kinase domain appears to be in database entries for CaMKIIδ splice variants: *e.g.*, Consensus Coding Sequence Database (CCDS) identifiers CCDS3703, which contains exon 6v1, and CCDS82948, which contains exon 6v2. Further studies are necessary to interrogate the structural details of these variants, as well as their biological relevance.

CaMKII exchanges subunits in an activation-dependent manner to form mixed oligomers (Stratton, Lee, Bhattacharyya, Christensen, Chao, Schulman, Groves, and Kuriyan, 2014; Bhattacharyya, Stratton, Going, McSpadden, Huang, Susa, Elleman, Cao, Pappireddi, Burkhardt, Gee, Barros,

This article is protected by copyright. All rights reserved

Schulman, Williams, and Kuriyan, 2016). It will be especially interesting to understand how the CaMKIIβ short hub variant affects subunit exchange properties (Stratton, Lee, Bhattacharyya, Christensen, Chao, Schulman, Groves, and Kuriyan, 2014). To date, the linker identity has not been shown to significantly affect subunit exchange rates in CaMKIIα (Stratton, Lee, Bhattacharyya, Christensen, Chao, Schulman, Groves, and Kuriyan, 2014). However, it will be important to test linker length differences in other CaMKII variants, as well as between CaMKII genes. CaMKIIα/β mixed oligomers are known to be abundant in the brain (Bennett, Erondu, and Kennedy, 1983; Kuret and Schulman, 1984; Miller and Kennedy, 1986), and it has been shown that CaMKIIα and β homo-oligomers undergo subunit exchange upon activation *in vitro* to form mixed oligomers (Bhattacharyya, Stratton, Going, McSpadden, Huang, Susa, Elleman, Cao, Pappireddi, Burkhardt, Gee, Barros, Schulman, Williams, and Kuriyan, 2016). Further experiments will be necessary to determine the effect of linker length/sequence on subunit exchange, and whether this could result in a specific population of mixed oligomers in specific cell types.

There are also clear effects of the linker composition on CaMKII structure. The kinases are physically attached to the hub domain through this variable linker, which is predicted to be intrinsically disordered. There have been many studies focused on understanding the structure of the holoenzyme, as well as the isolated hub and kinase domains (recently reviewed in (Bhattacharyya, Karandur, and Kuriyan, 2019)). There is evidence from negative stain electron microscopy (EM) studies that the kinases extend far from the hub domain, presumably not interacting with the hub (Morris and Török, 2001; Myers, Zaegel, Coultrap, Miller, Bayer, and Reichow, 2017). Alternatively, there is evidence from negative stain EM (Kolodziej, Hudmon, Waxham, and Stoops, 2000), small angle x-ray scattering (SAXS) and X-ray crystallography (Chao, Stratton, Lee, Rosenberg, Levitz, Mandell, Kortemme, Groves, Schulman, and Kuriyan, 2011), and cryo-EM (Sloutsky, Dziedzic, Dunn, Bates, Torres-Ocampo, Boopathy, Page, Weeks, Chao, and Stratton, 2020) experiments that indicate that the kinase domains do directly interact with the hub domain. SAXS experiments revealed that CaMKIIα-0 (zero residue linker) holoenzymes have radii consistent with the conformation in the X-ray crystal structure of CaMKIIα-0 in which all kinases are docked onto the hub domain (Chao, Stratton, Lee, Rosenberg, Levitz, Mandell, Kortemme, Groves, Schulman, and Kuriyan, 2011) (pdb: 3SOA). Cryo-EM reconstructions of CaMKIIα(15,18) showed evidence for this same docked conformation as well

This article is protected by copyright. All rights reserved

as an alternative docked conformation (Sloutsky, Dziedzic, Dunn, Bates, Torres-Ocampo, Boopathy, Page, Weeks, Chao, and Stratton, 2020). These data together suggest that the kinases interact with the hub domain both in the absence of a linker and in the presence a 30-residue linker. This stands to reason – since these two domains have evolved together, it would be surprising had they not also evolved ways to interact (Kuriyan and Eisenberg, 2007). Future experiments should further address the details of these specific interactions and their relevance to activity regulation and function.

To date, there is no structural information on the linker itself or how it may interact with the kinase or hub domains. Nonetheless, linker length and exon composition likely impact the dynamics within the holoenzyme structure, including the affinity of interactions between the kinase and the hub domains. In the crystal structure of the CaMKIIα holoenzyme, the variable linker region is completely deleted (Chao, Stratton, Lee, Rosenberg, Levitz, Mandell, Kortemme, Groves, Schulman, and Kuriyan, 2011). In cryo-EM reconstructions of CaMKIIα(14,18), the kinase domains adopted at least two different conformations in which the kinase directly interacts with the hub domain (Sloutsky, Dziedzic, Dunn, Bates, Torres-Ocampo, Boopathy, Page, Weeks, Chao, and Stratton, 2020). One was the same docked conformation as the one seen in the crystal structure, where the regulatory segment interacts with the hub domain (Chao, Stratton, Lee, Rosenberg, Levitz, Mandell, Kortemme, Groves, Schulman, and Kuriyan, 2011) (PDB: 3SOA), while the other docked conformation showed the kinase C-lobe (helices αEF and αG) interacting with the hub domain. Further studies will be needed to determine the functional validity of these docked conformations.

Impact of linker on CaMKII activity regulation

Consistent with kinase-docked conformation of CaMKIIα-0 in the solved crystal structure (Chao, Stratton, Lee, Rosenberg, Levitz, Mandell, Kortemme, Groves, Schulman, and Kuriyan, 2011), the sensitivity of CaMKIIα to activation by Ca^{2+}/CaM is correlated to the length of the variable linker. Purified CaMKIIα-0 requires significantly more Ca^{2+}/CaM for activation compared to CaMKIIα(14,18) (30-residue linker) and CaMKIIα(14,15,18) (41-residue linker) (Sloutsky, Dziedzic, Dunn, Bates, Torres-Ocampo, Boopathy, Page, Weeks, Chao, and Stratton, 2020; Chao, Stratton, Lee, Rosenberg, Levitz, Mandell, Kortemme, Groves, Schulman, and Kuriyan, 2011). However, this linker dependence appears to be context-sensitive, as variants CaMKIIβ ranging in linker length from 217 to

This article is protected by copyright. All rights reserved

Accepted Article

0 residues are activated by the same concentration of Ca^{2+}/CaM as CaMKIIα(14,18) and CaMKIIα(14,15,18). In addition, Bhattacharyya *et al.* (Bhattacharyya, Lee, Muratcioglu, Qiu, Nyayapati, Schulman, Groves, and Kuriyan, 2020) recently reported that CaMKIIα(14,18) (30-residue linker) acquires Thr286 phosphorylation more readily than Thr305/306 phosphorylation, while the opposite is true for CaMKIIβ(13,14a,16,17,18,19,20,21) (217-residue linker). Together, these data suggest that linker length/sequence may also affect the balance of activation and inhibition of CaMKII.

Concluding remarks

The intricacies of CaMKII structure and function have been the focus of many studies for the past ~40 years. There have been significant strides forward in understanding details of CaMKII structure, its downstream targets, and the enormous diversity of CaMKII variants produced from alternative splicing. However, many questions remain to be answered. Sequencing experiments have illuminated the diversity of CaMKII transcripts that exist in a single tissue, and this should be performed in all tissues where CaMKII is expressed. The levels of protein produced from these transcripts are currently unclear, which will be an important question to answer moving forward. Further understanding if and why so many CaMKII variants are necessary for function will be crucial to fully elucidating the role of this kinase in memory formation. Finally, a key area of future study is to pursue the effect of linker post-translational modifications on CaMKII structure and function.

Figure captions

Figure 1: Exon architecture of CaMKII. (A) One subunit of CaMKII highlighting exon boundaries in the kinase domain, regulatory segment and hub domain. Exon boundaries are mapped onto a subunit of the holoenzyme crystal structure in ribbon diagram (pdb:3SOA). Colors match the linear depiction above. There is no variable linker in this structure; the black dashed line indicates where the linker would be. (B) Exon boundaries are mapped onto a ribbon diagram for a tetramer of the hub domain (pdb:2UXO). Highlighted below is exon 23, which is alternatively spliced only in CaMKIIβ. The 26

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

residues that are spliced out of exon 23 are not included in the surface representation of the hub domain, highlighting a short helix and two beta turns that would be missing in this splice variant.

Figure 2: (A) Alternative splicing in the CaMKII variable linker region. All exon boundaries are shown where there is alternative splicing in the variable linker region. For simplicity, only the exons flanking the variable linker (exons 12 and 22) are shown. Nucleotides comprising codons at exon junctures are shown in red letters. Only the extreme 5' and 3' bases are denoted for each exon. Exons colored with hashed lines indicate internal splice sites, with the corresponding a (top) or b (bottom) nucelotides. (B) Linker splicing rules imposed by codons at the 5' and 3' ends of linker exons. Splicing of incompatible exons results in a frameshift. Arrows indicate compatible exon junctions. Any path from exon 12 to exon 22 along compatible exon junctions constitutes an in-frame splice variant. Every linker must contain exons 14 and 18 in order for exons to be translated in frame, with the exception of a junction between exons 13 (CaMKIIβ and γ) and 22, indicated by the dashed arrow, which also maintains exon 22 in frame.

Figure 3: Timeline of key events in the discovery of CaMKII and its splice variants. References: (1) (Yamauchi and Fujisawa, 1980); (2) (Payne and Soderling, 1980); (3) (Kennedy and Greengard, 1981); (4) (Woodgett et al., 1982); (5) (Bennett et al., 1983); (6) (Woodgett et al., 1983); (7) (McGuinness et al., 1983a); (8) (Woodgett et al., 1984); (9) (Bennett and Kennedy, 1987); (10) (Lin et al., 1987); (11) (Hanley et al., 1987); (12) (Bulleit et al., 1988); (13) (Tobimatsu et al., 1988); (14) (Tobimatsu and Fujisawa, 1989); (15) (Karls et al., 1992); (16) (Olson et al., 1995); (17) (Nishioka et al., 1996); (18) (Tombes et al., 2003); (*,#) see Fig. 5 for all references in which new CaMKII splice variants were introduced.

Figure 4: Linker exon sequences for all CaMKII genes. Residues highlighted in red are partially encoded in the preceding exon (due to split codons, see Fig. 2). Residues highlighted in blue are partially encoded in the subsequent exon. Residues in parentheses indicate that the identity of that residue is conditional on the preceding or subsequent exon. Residues highlighted in gray are spliced out due to internal splice sites (14b, 16b, 18b, 19b).

Figure 5: Historical designations of CaMKII variants. Sequences compiled for all four CaMKII

This article is protected by copyright. All rights reserved

genes, including the name the variant was given in the corresponding citation. Under 'linker exons' are all exons that are alternatively spliced. Question marks indicate ambiguity from the original reference due to lack of sequencing in the corresponding region. Hashed lines in exons indicate an internal splice site, where 'b' indicates the short version truncated at the internal splice site. References: (1) (Lin et al., 1987); (2) (Bennett and Kennedy, 1987); (3) (Bulleit et al., 1988); (4) (Tobimatsu et al., 1988); (5) (Tobimatsu and Fujisawa, 1989); (6) (Benson et al., 1991); (7) (Nghiem et al., 1993); (8) (Schworer et al., 1993); (9) (Mayer et al., 1993); (10) (Mayer et al., 1994b); (11) (Edman and Schulman, 1994); (12) (Zhou and Ikebe, 1994); (13) (Urquidi and Ashcroft, 1995); (14) (Brocke et al., 1995); (16) (Kwiatkowski and McGill, 1995); (17) (Mayer et al., 1995); (18) (Tombes and Krystal, 1997); (19) (Singer et al., 1997); (20) (Bayer et al., 1998); (21) (Takeuchi and Fujisawa, 1998); (22) (Bayer et al., 1999); (23) (Hoch et al., 1999); (24) (Takeuchi et al., 1999); (25) (Kwiatkowski and McGill, 2000); (26) (Wang et al., 2000); (27) (Takeuchi et al., 2000); (29) (Gangopadhyay et al., 2003); (30) (Chang et al., 2009); (31) (Cook et al., 2018)

Acknowledgements. Thanks to members of the Stratton lab for helpful discussions. This work was supported by a grant to MMS: R01GM123157.

Conflicts of interest: The authors declare no conflicts of interest.

Abbreviations:

Ca^{2+}/CaM (calcium/calmodulin)

CaMKII (Ca^{2+}/CaM dependent protein kinase II)

CaMKAP (CaMKII associated protein)

EM (electron microscopy)

LTCC (L-type Ca^{2+} channel)

NLS (nuclear localization sequence)

This article is protected by copyright. All rights reserved

Accepted Article

SAXS (small angle X-ray scattering)

References

Z. Ahmad, A. A. DePaoli-Roach, and P. J. Roach. Purification and characterization of a rabbit liver calmodulin- dependent protein kinase able to phosphorylate glycogen synthase. (1982) J Biol Chem, **257**(14):8348–55

Johannes Backs, Thea Backs, Stefan Neef, Michael M. Kreusser, Lorenz H. Lehmann, David M. Patrick, Chad E. Grueter, Xiaoxia Qi, James A. Richardson, Joseph A. Hill, Hugo A. Katus, Rhonda Bassel-Duby, Lars S. Maier, and Eric N. Olson. The delta isoform of cam kinase ii is required for pathological cardiac hypertrophy and remodeling after pressure overload. (2009) Proc Natl Acad Sci U S A, **106**(7):2342–7

Alaji Bah and Julie D. Forman-Kay. (2016) Modulation of intrinsically disordered protein function by post-translational modifications. J Biol Chem, **291**(13):6696–705

Anthony J. Baucum, Brian C. Shonesy, Kristie L. Rose, and Roger J. Colbran. (2015) Quantitative proteomics analysis of camkii phosphorylation and the camkii interactome in the mouse forebrain. ACS Chem Neurosci, **6**(4):615–31

K. U. Bayer, K. Harbers, and H. Schulman. (1998) alphakap is an anchoring protein for a novel cam kinase ii isoform in skeletal muscle. EMBO J, **17**(19):5598–605

K. U. Bayer, J. Löhler, H. Schulman, and K. Harbers. (1999) Developmental expression of the cam kinase ii isoforms: ubiquitous gamma- and delta-cam kinase ii are the early isoforms and most abundant in the developing nervous system. Brain Res Mol Brain Res, **70**(1):147–54

M. K. Bennett and M. B. Kennedy. (1987) Deduced primary structure of the beta subunit of brain type ii ca2+/calmodulin- dependent protein kinase determined by molecular cloning. Proc Natl Acad Sci U S A, **84**(7):1794–8

Accepted Article

This article is protected by copyright. All rights reserved

M. K. Bennett, N. E. Erondu, and M. B. Kennedy. (1983) Purification and characterization of a calmodulin-dependent protein kinase that is highly concentrated in brain. J Biol Chem, **258**(20):12735–44

D. L. Benson, P. J. Isackson, C. M. Gall, and E. G. Jones. (1991) Differential effects of monocular deprivation on glutamic acid decarboxylase and type ii calcium-calmodulin-dependent protein kinase gene expression in the adult monkey visual cortex. J Neurosci, **11**(1):31–47

Moitrayee Bhattacharyya, Margaret M. Stratton, Catherine C. Going, Ethan D. McSpadden, Yongjian Huang, Anna C. Susa, Anna Elleman, Yumeng Melody Cao, Nishant Pappireddi, Pawel Burkhardt, Christine L. Gee, Tiago Barros, Howard Schulman, Evan R. Williams, and John Kuriyan. (2016) Molecular mechanism of activation-triggered subunit exchange in ca(2+)/calmodulin-dependent protein kinase ii. Elife, **5**

Moitrayee Bhattacharyya, Deepti Karandur, and John Kuriyan. Structural insights into the regulation of ca2+/calmodulin- dependent protein kinase ii (camkii). Cold Spring Harb Perspect Biol, 2019. ISSN 1943-0264. doi: 10.1101/ cshperspect.a035147.

Moitrayee Bhattacharyya, Young Kwang Lee, Serena Muratcioglu, Baiyu Qiu, Priya Nyayapati, Howard Schulman, Jay T. Groves, and John Kuriyan. (2020) Flexible linkers in camkii control the balance between activating and inhibitory autophosphorylation. Elife, **9**

D. K. Blumenthal, K. Takio, A. M. Edelman, H. Charbonneau, K. Titani, K. A. Walsh, and E. G. Krebs. (1985) Identification of the calmodulin-binding domain of skeletal muscle myosin light chain kinase. Proc Natl Acad Sci U S A, **82**(10): 3187–91

L. Brocke, M. Srinivasan, and H. Schulman. (1995) Developmental and regional expression of multifunctional ca2+/calmodulin- dependent protein kinase isoforms in rat brain. J Neurosci, **15**(10):6797–808

R. F. Bulleit, M. K. Bennett, S. S. Molloy, J. B. Hurley, and M. B. Kennedy. (1988) Conserved and variable regions in the subunits of brain type ii ca2+/calmodulin-dependent protein kinase. Neuron,

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

1(1):63–72

Heng-Yu Y. Chang, Kyra Minahan, Julie A. Merriman, and Keith T. Jones. (2009) Calmodulin-dependent protein kinase gamma 3 (camkiigamma3) mediates the cell cycle resumption of metaphase ii eggs in mouse. Development, **136**(24):4077–81

Luke H. Chao, Margaret M. Stratton, Il-Hyung H. Lee, Oren S. Rosenberg, Joshua Levitz, Daniel J. Mandell, Tanja Kortemme, Jay T. Groves, Howard Schulman, and John Kuriyan. (2011) A mechanism for tunable autoinhibition in the structure of a human ca2+/calmodulin- dependent kinase ii holoenzyme. Cell, **146**(5):732–45

W. Y. Cheung. (1970) Cyclic 3',5'-nucleotide phosphodiesterase. demonstration of an activator. Biochem Biophys Res Commun, **38**(3):533–8

W. Y. Cheung. (1971) Cyclic 3',5'-nucleotide phosphodiesterase. evidence for and properties of a protein activator. J Biol Chem, **246**(9):2859–69

W. Y. Cheung. (1980) Calmodulin plays a pivotal role in cellular regulation. Science, **207**(4426):19–27

Philip Cohen, Ann Burchell, J. Gordon Foulkes, Patricia T.W Cohen, Thomas C. Vanaman, and Anagus C. Nairin. (1978) Identification of the ca2+dependent modulator protein as the fourth subunit of rabbit skeletal muscle phosphorylase kinase. FEBS Letters, **92**(2):287–293

Roger J. Colbran. (2004) Targeting of calcium/calmodulin-dependent protein kinase ii. Biochem J, **378**(Pt 1):1–16

Sarah G. Cook, Ashley M. Bourke, Heather O'Leary, Vincent Zaegel, Erika Lasda, Janna Mize-Berge, Nidia Quillinan, Chandra L. Tucker, Steven J. Coultrap, Paco S. Herson, and K. Ulrich Bayer. (2018) Analysis of the camkiiα and β splice-variant distribution among brain regions reveals isoform-specific differences in holoenzyme formation. Sci Rep, **8**(1):5448

R. Dabrowska, J. M. Sherry, D. K. Aromatorio, and D. J. Hartshorne. (1978) Modulator protein as a

This article is protected by copyright. All rights reserved

component of the myosin light chain kinase from chicken gizzard. Biochemistry, **17**(2):253–8

A. M. Edelman, D. K. Blumenthal, and E. G. Krebs. (1987) Protein serine/threonine kinases. Annu Rev Biochem, **56**:567–613

C. F. Edman and H. Schulman. (1994) Identification and characterization of delta b-cam kinase and delta c-cam kinase from rat heart, two new multifunctional ca2+/calmodulin-dependent protein kinase isoforms. Biochim Biophys Acta, **1221**(1):89–101

Ype Elgersma, Nikolai B. Fedorov, Sami Ikonen, Esther S. Choi, Minetta Elgersma, Ofelia M. Carvalho, Karl Peter Giese, and Alcino J. Silva. (2002) Inhibitory autophosphorylation of camkii controls psd association, plasticity, and learning. Neuron, **36**(3):493–505

Jessica Escoffier, Hoi Chang Lee, Sandra Yassine, Raoudha Zouari, Guillaume Martinez, Thomas Karaouzène, Charles Coutton, Zine-Eddine E. Kherraf, Lazhar Halouani, Chema Triki, Serge Nef, Nicolas Thierry-Mieg, Sergey N. Savinov, Rafael Fissore, Pierre F. Ray, and Christophe Arnoult. (2016) Homozygous mutation of plcz1 leads to defective human oocyte activation and infertility that is not rescued by the ww-binding protein pawp. Hum Mol Genet, **25**(5): 878–91

K. Fukunaga, H. Yamamoto, K. Matsui, K. Higashi, and E. Miyamoto. (1982) Purification and characterization of a ca2+- and calmodulin-dependent protein kinase from rat brain. J Neurochem, **39**(6):1607–17

Samudra S. Gangopadhyay, Amy L. Barber, Cynthia Gallant, Zenon Grabarek, Janet L. Smith, and Kathleen G. Morgan. (2003) Differential functional properties of calmodulin-dependent protein kinase iigamma variants isolated from smooth muscle. Biochem J, **372**(Pt 2):347–57

Jianjiong Gao and Dong Xu. (2012) Correlation between posttranslational modification and intrinsic disorder in protein. Pac Symp Biocomput, pages 94–103

K. P. Giese, N. B. Fedorov, R. K. Filipkowski, and A. J. Silva. (1998) Autophosphorylation at thr286 of the alpha calcium- calmodulin kinase ii in ltp and learning. Science, **279**(5352):870–3

Accepted Article

This article is protected by copyright. All rights reserved

J. R. Goldenring, B. Gonzalez, J. S. McGuire, and R. J. DeLorenzo. (1983) Purification and characterization of a calmodulin- dependent kinase from rat brain cytosol able to phosphorylate tubulin and microtubule-associated proteins. J Biol Chem, **258**(20):12632–40

R. M. Hanley, A. R. Means, T. Ono, B. E. Kemp, K. E. Burgin, N. Waxham, and P. T. Kelly. (1987) Functional analysis of a complementary dna for the 50-kilodalton subunit of calmodulin kinase ii. Science, **237**(4812):293–7

E. K. Heist, M. Srinivasan, and H. Schulman. (1998) Phosphorylation at the nuclear localization signal of ca2+/calmodulin- dependent protein kinase ii blocks its nuclear targeting. J Biol Chem, **273**(31):19763–71

Bruce E. Herring and Roger A. Nicoll. (2016) Long-term potentiation: From camkii to ampa receptor trafficking. Annu Rev Physiol, **78**:351–65

B. Hoch, R. Meyer, R. Hetzer, E. G. Krause, and P. Karczewski. (1999) Identification and expression of delta-isoforms of the multifunctional ca2+/calmodulin-dependent protein kinase in failing and nonfailing human myocardium. Circ Res, **84**(6):713–21

Salvatore Incontro, Javier Díaz-Alonso, Jillian Iafrati, Marta Vieira, Cedric S. Asensio, Vikaas S. Sohal, Katherine W. Roche, Kevin J. Bender, and Roger A. Nicoll. (2018) The camkii/nmda receptor complex controls hippocampal synaptic transmission by kinase-dependent and independent mechanisms. Nat Commun, **9**(1):2069

T. Iwasa, K. Fukunaga, H. Yamamoto, E. Tanaka, and E. Miyamoto. (1983) Ca2+, calmodulin-dependent phosphorylation of glycogen synthase by a brain protein kinase. FEBS Lett, **161**(1):28–32

Peter Jehl, Jean Manguy, Denis C. Shields, Desmond G. Higgins, and Norman E. Davey. (2016) Proviz-a web-based visualization tool to investigate the functional and evolutionary features of protein sequences. Nucleic Acids Res, **44**(W1):W11–5

Shiro Kakiuchi and Reiko Yamazaki. (1970) Calcium dependent phosphodiesterase activity and its activating factor (paf) from brain: Studies on cyclic 3,5-nucleotide phosphodiesterase (iii).

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Biochemical and Biophysical Research Communications, **41**(5):1104–1110

Shiro KAKIUCHI, Reiko YAMAZAKI, and Hisashi NAKAJIMA. (1970) Properties of a heat-stable phosphodiesterase activating factor isolated from brain extract studies on cyclic 3', 5'-nucleotide phosphodiesterase. ii. Proceedings of the Japan Academy, **46**(6):587–592

U. Karls, U. Müller, D. J. Gilbert, N. G. Copeland, N. A. Jenkins, and K. Harbers. (1992) Structure, expression, and chromosome location of the gene for the beta subunit of brain-specific ca2+/calmodulin-dependent protein kinase ii identified by transgene integration in an embryonic lethal mouse mutant. Mol Cell Biol, **12**(8):3644–52

P. T. Kelly, T. L. McGuinness, and P. Greengard. (1984) Evidence that the major postsynaptic density protein is a component of a ca2+/calmodulin-dependent protein kinase. Proc Natl Acad Sci U S A, **81**(3):945–9

M. B. Kennedy and P. Greengard. (1981) Two calcium/calmodulin-dependent protein kinases, which are highly concentrated in brain, phosphorylate protein i at distinct sites. Proc Natl Acad Sci U S A, **78**(2):1293–7

M. B. Kennedy, T. McGuinness, and P. Greengard. (1983) A calcium/calmodulin-dependent protein kinase from mammalian brain that phosphorylates synapsin i: partial purification and characterization. J Neurosci, **3**(4):818–31

Shahid Khan, Kenneth H. Downing, and Justin E. Molloy. (2019) Architectural dynamics of camkii-actin networks. Biophys J, **116**(1):104–119

S. J. Kolodziej, A. Hudmon, M. N. Waxham, and J. K. Stoops. (2000) Three-dimensional reconstructions of calcium/calmodulin- dependent (cam) kinase iialpha and truncated cam kinase iialpha reveal a unique organization for its structural core and functional domains. J Biol Chem, **275**(19):14354–9

J. Kuret and H. Schulman. (1984) Purification and characterization of a ca2+/calmodulin-dependent protein kinase from rat brain. Biochemistry, **23**(23):5495–504

This article is protected by copyright. All rights reserved

Accepted Article

John Kuriyan and David Eisenberg. (2007) The origin of protein interactions and allostery in colocalization. Nature, **450**(7172):983–990

A. P. Kwiatkowski and J. M. McGill. (1995) Human biliary epithelial cell line mz-cha-1 expresses new isoforms of calmodulin-dependent protein kinase ii. Gastroenterology, **109**(4):1316–23

A. P. Kwiatkowski and J. M. McGill. (2000) Alternative splice variant of gamma-calmodulin-dependent protein kinase ii alters activation by calmodulin. Arch Biochem Biophys, **378**(2):377–83

Y. L. Lai, T.L. M. McGuinness, and P. G. Greengard. (1983) Purification and characterization of brain ca2+ /calmodulin- dependent protein kinase ii that phosphorylates synapsin i., Soc. Neurosci. Abstr., **9**, 1029

C. R. Lin, M. S. Kapiloff, S. Durgerian, K. Tatemoto, A. F. Russo, P. Hanson, H. Schulman, and M. G. Rosenfeld. (1987) Molecular cloning of a brain-specific calcium/calmodulin-dependent protein kinase. Proc Natl Acad Sci U S A, **84**(16):5962–6

T. J. Lukas, W. H. Burgess, F. G. Prendergast, W. Lau, and D. M. Watterson. (1986) Calmodulin binding domains: charac- terization of a phosphorylation and calmodulin binding site from myosin light chain kinase. Biochemistry, **25**(6): 1458–64

Huan Ma, Rachel D. Groth, Samuel M. Cohen, John F. Emery, Boxing Li, Esthelle Hoedt, Guoan Zhang, Thomas A. Neubert, and Richard W. Tsien. (2014) γcamkii shuttles ca^{2+}/cam to the nucleus to trigger creb phosphorylation and gene expression. Cell, **159**(2):281–94

Huan Ma, Boxing Li, and Richard W. Tsien. (2015) Distinct roles of multiple isoforms of camkii in signaling to the nucleus. Biochim Biophys Acta, **1853**(9):1953–7

Lars S. Maier and Donald M. Bers. (2002) Calcium, calmodulin, and calcium-calmodulin kinase ii: heartbeat to heartbeat and beyond. J Mol Cell Cardiol, **34**(8):919–39

P. Mayer, M. Möhlig, H. Schatz, and A. Pfeiffer. (1993) New isoforms of multifunctional calcium/calmodulin-dependent protein kinase ii. FEBS Lett, **333**(3):315–8

This article is protected by copyright. All rights reserved

Accepted Article

P. Mayer, M. Möhlig, H. Schatz, and A. Pfeiffer. (1994) Additional isoforms of multifunctional calcium/calmodulin-dependent protein kinase ii in rat heart tissue. Biochem J, **298**(Pt 3):757–8

P. Mayer, M. Möhlig, U. Seidler, H. Rochlitz, M. Fährmann, H. Schatz, H. Hidaka, and A. Pfeiffer. (1994) Characterization of gamma- and delta-subunits of ca2+/calmodulin-dependent protein kinase ii in rat gastric mucosal cell populations. Biochem J, **297**(Pt 1):157–62

P. Mayer, M. Möhlig, D. Idlibe, and A. Pfeiffer. (1995) Novel and uncommon isoforms of the calcium sensing enzyme calcium/calmodulin dependent protein kinase ii in heart tissue. Basic Res Cardiol, **90**(5):372–9

T. L. McGuinness, Y. Lai, P. Greengard, J. R. Woodgett, and P. Cohen. (1983) A multifunctional calmodulin-dependent protein kinase. similarities between skeletal muscle glycogen synthase kinase and a brain synapsin i kinase. FEBS Lett, **163**(2):329–34

T.L. M. McGuinness, P.T. K. Kelly, C.C. O. Ouimet, and P. G. Greengard. (1983) Studies on the subcellular and regional distribution of calmodulin-dependent protein kinase ii in rat brain., Soc. Neurosci. Abstr., **9**, 1029.

S. G. Miller and M. B. Kennedy. (1985) Distinct forebrain and cerebellar isozymes of type ii ca2+/calmodulin-dependent protein kinase associate differently with the postsynaptic density fraction. J Biol Chem, **260**(15):9039–46

S. G. Miller and M. B. Kennedy. (1986) Regulation of brain type ii ca2+/calmodulin-dependent protein kinase by au- tophosphorylation: a ca2+-triggered molecular switch. Cell, **44**(6):861–70

E. P. Morris and K. Török. (2001) Oligomeric structure of alpha-calmodulin-dependent protein kinase ii. J Mol Biol, **308**(1): 1–8

Janette B. Myers, Vincent Zaegel, Steven J. Coultrap, Adam P. Miller, K. Ulrich Bayer, and Steve L. Reichow. (2017) The camkii holoenzyme structure in activation-competent conformations. Nat Commun, **8**:15742

This article is protected by copyright. All rights reserved

A. C. Nairn and P. Greengard. (1987) Purification and characterization of ca2+/calmodulin-dependent protein kinase i from bovine brain. J Biol Chem, **262**(15):7273–81

A. C. Nairn and S. V. Perry. (1979) Calmodulin and myosin light-chain kinase of rabbit fast skeletal muscle. Biochem J, **179**(1):89–97

A. C. Nairn, B. Bhagat, and H. C. Palfrey. (1985) Identification of calmodulin-dependent protein kinase iii and its major mr 100,000 substrate in mammalian tissues. Proc Natl Acad Sci U S A, **82**(23):7939–43

A.C. Nairn and P. Greengard. (1984) Widespread tissue distribution of ca2+/calmodulin-dependent protein kinase i that phosphorylates synapsin 1., Fed. Proc. Fed. Am. Soc. Exp. Biol., **43**(6-8), 1467.

A.C. N. Nairn and P. G. Greengard. (1983) Purification and characterization of brain ca2+/calmodulin-dependent protein kinase i that phosphorylates synapsin i., Soc. Neurosci. Abstr., **9**, 1029

Eric J. Nestler and Paul Greengard. Protein phosphorylation in the nervous system. Wiley, 1984. ISBN 0471805580.

P. Nghiem, S. M. Saati, C. L. Martens, P. Gardner, and H. Schulman. (1993) Cloning and analysis of two new isoforms of multifunctional ca2+/calmodulin-dependent protein kinase. expression in multiple human tissues. J Biol Chem, **268**(8):5471–9

N. Nishioka, M. Shiojiri, S. Kadota, H. Morinaga, J. Kuwahara, T. Arakawa, S. Yamamoto, and T. Yamauchi. (1996) Gene of rat ca2+/calmodulin-dependent protein kinase ii alpha isoform – its cloning and whole structure. FEBS Lett, **396**(2-3):333–6

Heather O'Leary, Erika Lasda, and K. Ulrich Bayer. (2006) Camkiibeta association with the actin cytoskeleton is regulated by alternative splicing. Mol Biol Cell, **17**(11):4656–65

N. J. Olson, T. Massé, T. Suzuki, J. Chen, D. Alam, and P. T. Kelly. (1995) Functional identification of the promoter for the gene encoding the alpha subunit of calcium/calmodulin-dependent protein

This article is protected by copyright. All rights reserved

Accepted Article

kinase ii. Proc Natl Acad Sci U S A, **92**(5):1659–63

H. C. Palfrey. (1983) Presence in many mammalian tissues of an identical major cytosolic substrate (mr 100 000) for calmodulin- dependent protein kinase. FEBS Lett, **157**(1):183–90

M. E. Payne and T. R. Soderling. (1980) Calmodulin-dependent glycogen synthase kinase. J Biol Chem, **255**(17):8054–6

M. E. Payne, C. M. Schworer, and T. R. Soderling. (1983) Purification and characterization of rabbit liver calmodulin-dependent glycogen synthase kinase. J Biol Chem, **258**(4):2376–82

A. J. Robison, Martha A. Bass, Yuxia Jiao, Leigh B. MacMillan, Leigh C. Carmody, Ryan K. Bartlett, and Roger J. Colbran. (2005) Multivalent interactions of calcium/calmodulin-dependent protein kinase ii with the postsynaptic density proteins nr2b, densin-180, and alpha-actinin-2. J Biol Chem, **280**(42):35329–36

H. Schulman and P. Greengard. (1978) Stimulation of brain membrane protein phosphorylation by calcium and an endogenous heat-stable protein. Nature, **271**(5644):478–9

H. Schulman and P. Greengard. (1978) Ca2+-dependent protein phosphorylation system in membranes from various tissues, and its activation by "calcium-dependent regulator". Proc Natl Acad Sci U S A, **75**(11):5432–6

C. M. Schworer, R. W. McClure, and T. R. Soderling. (1985) Calmodulin-dependent protein kinases purified from rat brain and rabbit liver. Arch Biochem Biophys, **242**(1):137–45

C. M. Schworer, L. I. Rothblum, T. J. Thekkumkara, and H. A. Singer. (1993) Identification of novel isoforms of the delta subunit of ca2+/calmodulin-dependent protein kinase ii. differential expression in rat brain and aorta. J Biol Chem, **268**(19):14443–9

S. Shenolikar, R. Lickteig, D. G. Hardie, T. R. Soderling, R. M. Hanley, and P. T. Kelly. (1986) Calmodulin-dependent multifunctional protein kinase. evidence for isoenzyme forms in mammalian tissues. Eur J Biochem, **161**(3):739–47

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Shirish SHENOLIKAR, Patricia T. W. COHEN, Philip COHEN, Angus C. NAIRN, and S. Victor PERRY. (1979) The role of calmodulin in the structure and regulation of phosphorylase kinase from rabbit skeletal muscle. European Journal of Biochemistry, **100**(2):329–337

H. A. Singer, H. A. Benscoter, and C. M. Schworer. (1997) Novel ca2+/calmodulin-dependent protein kinase ii gamma-subunit variants expressed in vascular smooth muscle, brain, and cardiomyocytes. J Biol Chem, **272**(14):9393–400

Roman Sloutsky, Noelle Dziedzic, Matthew J. Dunn, Rachel M. Bates, Ana P. Torres-Ocampo, Sivakumar Boopathy, Brendan Page, John G. Weeks, Luke H. Chao, and Margaret M. Stratton. Heterogeneity in human hippocampal camkii transcripts reveals allosteric hub-dependent regulation. bioRxiv, page 721589, 1 2020. doi: 10.1101/721589.

Margaret Stratton, Il-Hyung H. Lee, Moitrayee Bhattacharyya, Sune M. Christensen, Luke H. Chao, Howard Schulman, Jay T. Groves, and John Kuriyan. (2014) Activation-triggered subunit exchange between camkii holoenzymes facilitates the spread of kinase activity. Elife, **3**:e01610

James T. Stull, Mary H. Nunnally, and Carolyn H. Michnoff. 4. Calmodulin-Dependent Protein Kinases, volume **17**, pages 113–166. Academic Press, 1 1986

Toru Suzuki, Emi Suzuki, Naoko Yoshida, Atsuko Kubo, Hongmei Li, Erina Okuda, Manami Amanai, and Anthony C. F. Perry. (2010) Mouse emi2 as a distinctive regulatory hub in second meiotic metaphase. Development, **137**(19):3281–91

M. Takeuchi and H. Fujisawa. (1998) New alternatively spliced variants of calmodulin-dependent protein kinase ii from rabbit liver. Gene, **221**(1):107–15

Y. Takeuchi, H. Yamamoto, K. Matsumoto, T. Kimura, S. Katsuragi, T. Miyakawa, and E. Miyamoto. (1999) Nuclear localization of the delta subunit of ca2+/calmodulin-dependent protein kinase ii in rat cerebellar granule cells. J Neurochem, **72**(2):815–25

Y. Takeuchi, H. Yamamoto, K. Fukunaga, T. Miyakawa, and E. Miyamoto. (2000) Identification of the isoforms of ca(2+)/calmodulin-dependent protein kinase ii in rat astrocytes and their subcellular

This article is protected by copyright. All rights reserved

localization. J Neurochem, **74**(6):2557–67

T. Tobimatsu and H. Fujisawa. (1989) Tissue-specific expression of four types of rat calmodulin-dependent protein kinase ii mrnas. J Biol Chem, **264**(30):17907–12

T. Tobimatsu, I. Kameshita, and H. Fujisawa. (1988) Molecular cloning of the cdna encoding the third polypeptide (gamma) of brain calmodulin-dependent protein kinase ii. J Biol Chem, **263**(31):16082–6

R. M. Tombes and G. W. Krystal. (1997) Identification of novel human tumor cell-specific camk-ii variants. Biochim Biophys Acta, **1355**(3):281–92

Robert M. Tombes, M. Omar Faison, and J. M. Turbeville. Organization and evolution of multifunctional ca(2+)/cam- dependent protein kinase genes. (2003) Gene, **322**:17–31

V. Urquidi and S. J. Ashcroft. (1995) A novel pancreatic beta-cell isoform of calcium/calmodulin-dependent protein kinase ii (beta 3 isoform) contains a proline-rich tandem repeat in the association domain. FEBS Lett, **358**(1):23–6

P. Wang, Y. L. Wu, T. H. Zhou, Y. Sun, and G. Pei. (2000) Identification of alternative splicing variants of the beta subunit of human ca(2+)/calmodulin-dependent protein kinase ii with different activities. FEBS Lett, **475**(2):107–10

Xiaohan Wang, Christian R. Marks, Tyler L. Perfitt, Terunaga Nakagawa, Amy Lee, David A. Jacobson, and Roger J. Colbran. (2017) A novel mechanism for ca2+/calmodulin-dependent protein kinase ii targeting to l-type ca2+channels that initiates long-range signaling to the nucleus. J Biol Chem, **292**(42):17324–17336

J. R. Woodgett, N. K. Tonks, and P. Cohen. (1982) Identification of a calmodulin-dependent glycogen synthase kinase in rabbit skeletal muscle, distinct from phosphorylase kinase. FEBS Lett, **148**(1):5–11

J. R. Woodgett, M. T. Davison, and P. Cohen. (1983) The calmodulin-dependent glycogen synthase kinase from rabbit skeletal muscle. purification, subunit structure and substrate specificity. Eur J

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Biochem, **136**(3):481–7

J. R. Woodgett, P. Cohen, T. Yamauchi, and H. Fujisawa. (1984) Comparison of calmodulin-dependent glycogen synthase kinase from skeletal muscle and calmodulin-dependent protein kinase-ii from brain. FEBS Lett, **170**(1):49–54

K. Yagi, M. Yazawa, S. Kakiuchi, M. Ohshima, and K. Uenishi. (1978) Identification of an activator protein for myosin light chain kinase as the ca2+-dependent modulator protein. J Biol Chem, **253**(5):1338–40

T. Yamauchi and H. Fujisawa. Evidence for three distinct forms of calmodulin-dependent protein kinases from rat brain. (1980) FEBS Lett, **116**(2):141–4

T. Yamauchi and H. Fujisawa. (1983) Purification and characterization of the brain calmodulin-dependent protein kinase (kinase ii), which is involved in the activation of tryptophan 5-monooxygenase. Eur J Biochem, **132**(1):15–21

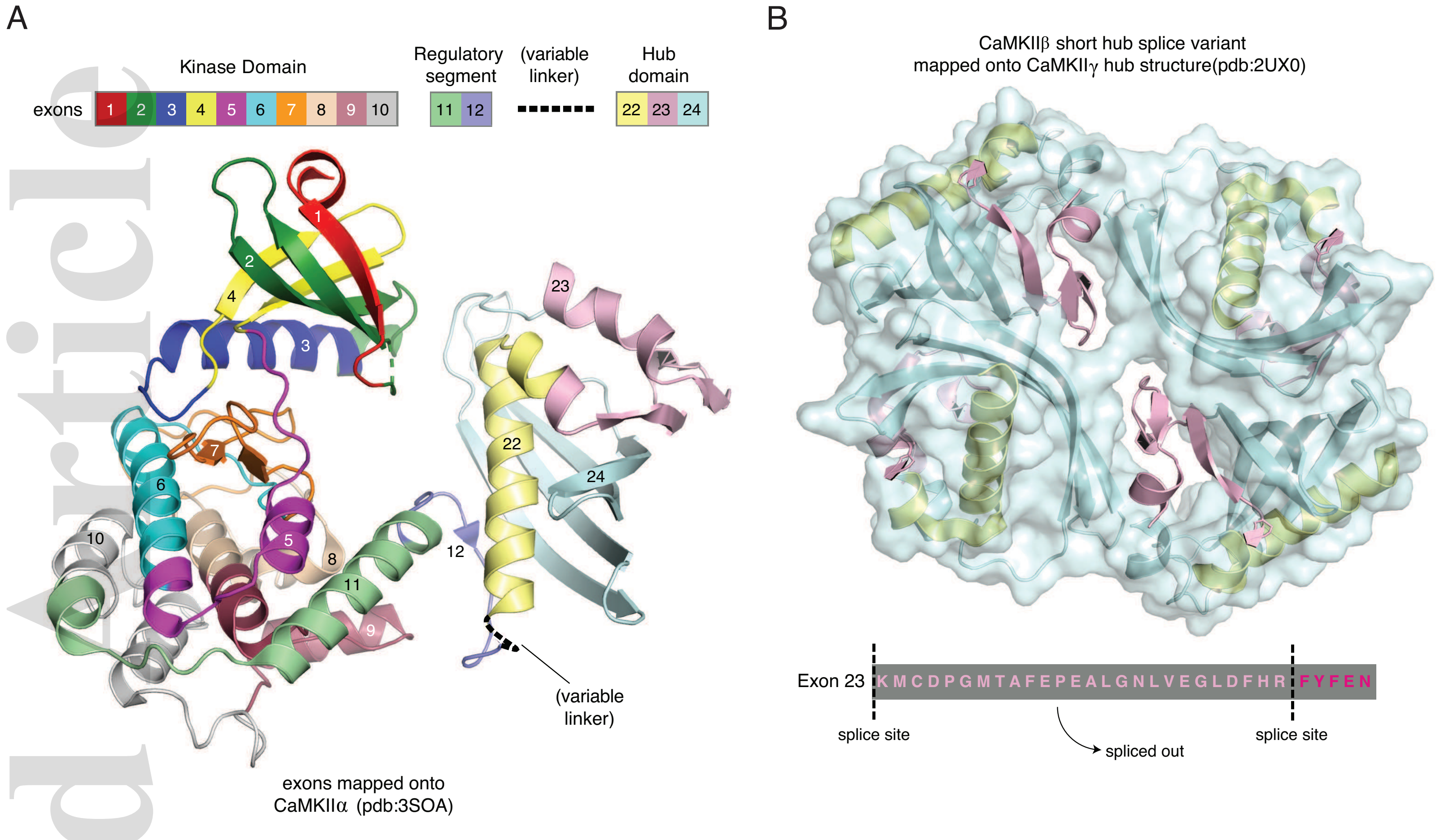
T. Yamauchi, H. Nakata, and H. Fujisawa. (1981) A new activator protein that activates tryptophan 5-monooxygenase and tyrosine 3-monooxygenase in the presence of ca2+-, calmodulin-dependent protein kinase. purification and characterization. J Biol Chem, **256**(11):5404–9

Takashi Y. Yamauchi and Hitoshi F. Fujisawa. Phosphorylation of microtubule-associated protein 2 by calmodulin- dependent protein kinase (kinase ii) which occurs only in the brain tissues. (1982) Biochemical and Biophysical Research Communications, **109**(3):975–981

Sook-Young Y. Yoon, Teru Jellerette, Ana Maria Salicioni, Hoi Chang Lee, Myung-Sik S. Yoo, Kevin Coward, John Parrington, Daniel Grow, Jose B. Cibelli, Pablo E. Visconti, Jesse Mager, and Rafael A. Fissore. Human sperm devoid of plc, zeta 1 fail to induce ca(2+) release and are unable to initiate the first step of embryo development. (2008) J Clin Invest, **118**(11):3671–81

Z. L. Zhou and M. Ikebe. New isoforms of ca2+/calmodulin-dependent protein kinase ii in smooth muscle. (1994) Biochem J, **299**(Pt 2):489–95

This article is protected by copyright. All rights reserved

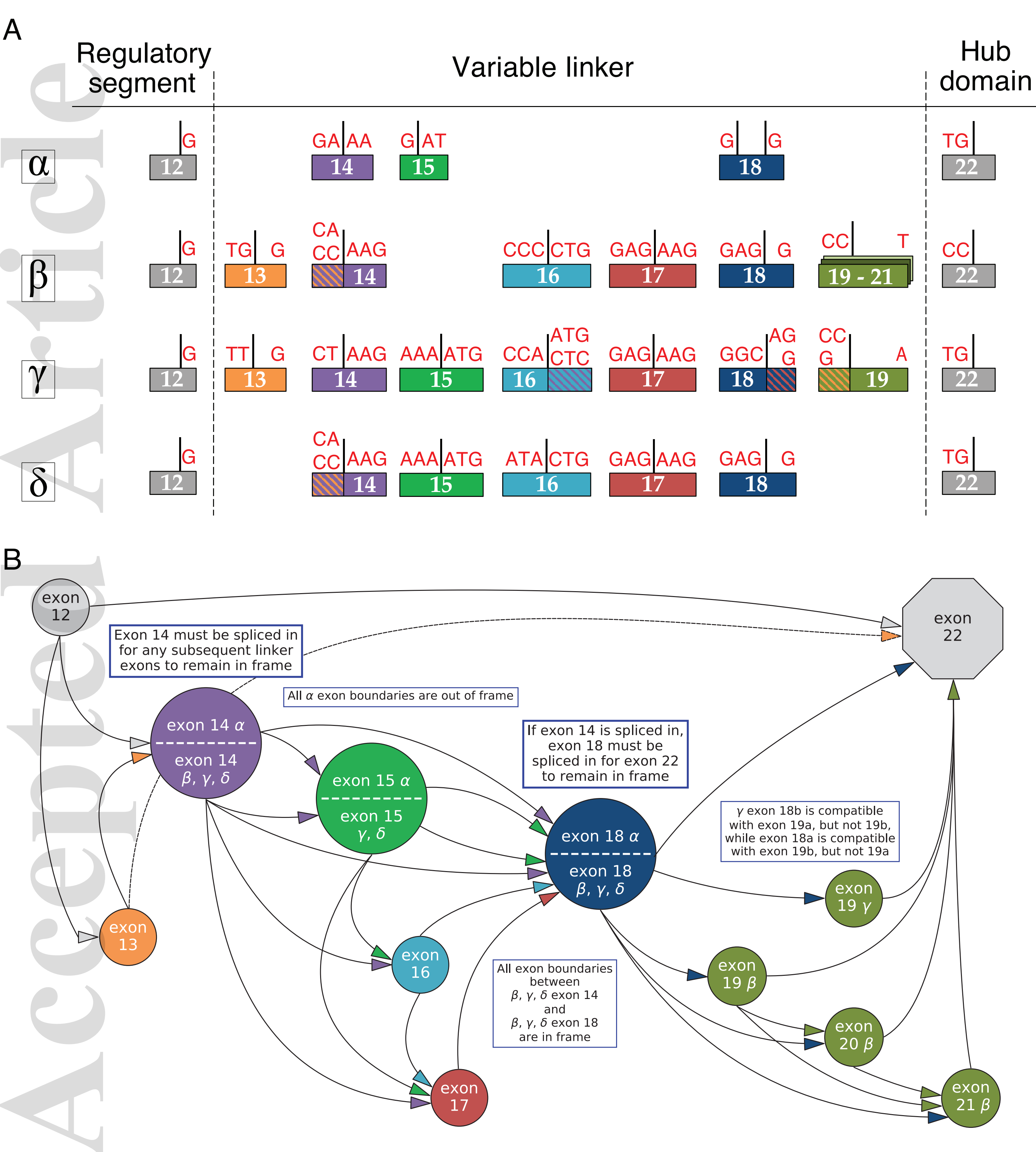


ejn_14761_f1.eps

Accepted Article

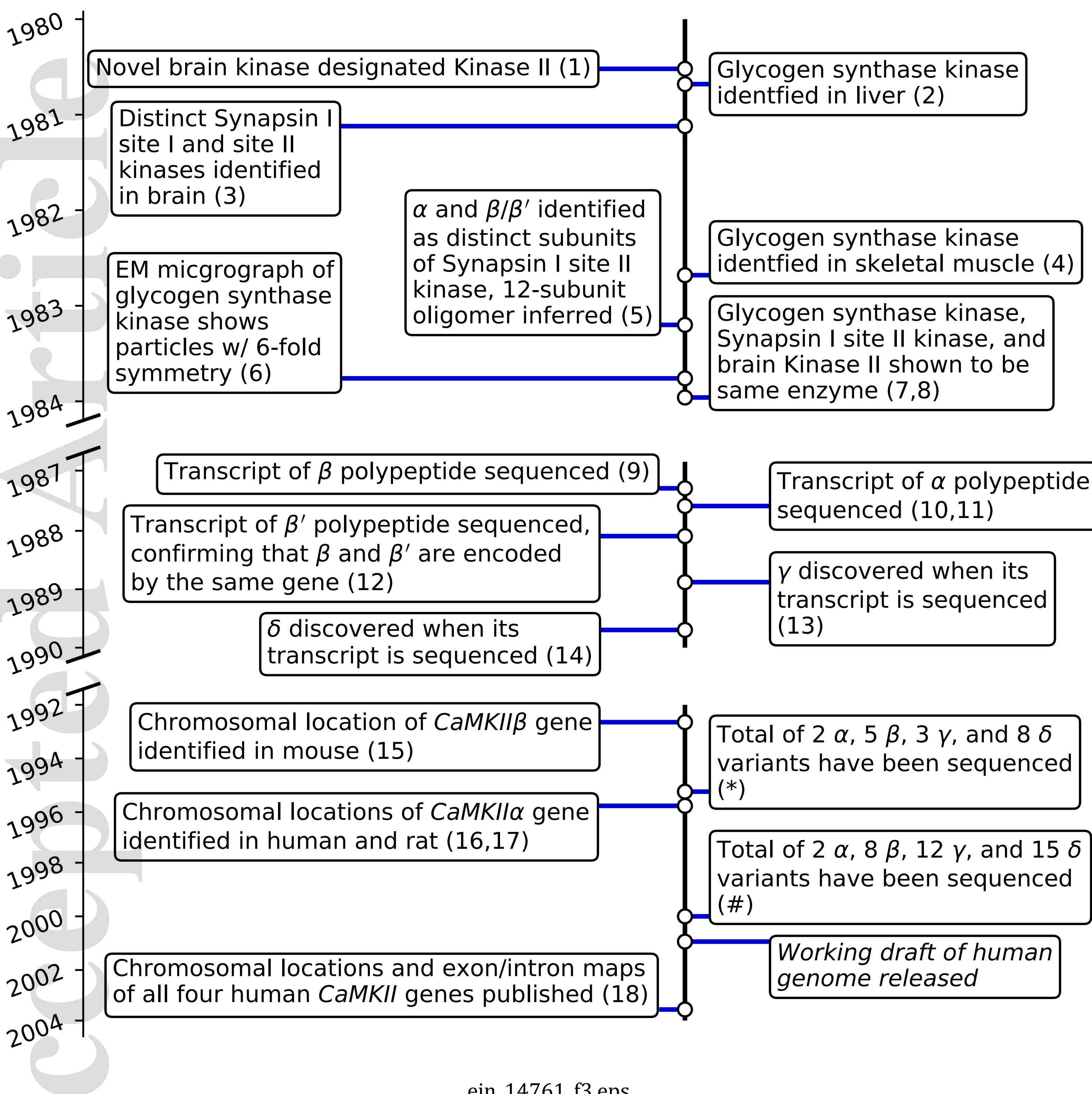
This article is protected by copyright. All rights reserved

Accepted Article



ejn_14761_f2.eps

This article is protected by copyright. All rights reserved



ejn_14761_f3.eps

Accepted Article

This article is protected by copyright. All rights reserved

13	14	15	16	17	18	19 - 21

Exon label	α sequence	β sequence	γ sequence	δ sequence
13	*not encoded in genome*	VGRQTTAPATMSTAASGTT MGLVEQA	VGRQSSAPASPAASAAGLAGQA	*not encoded in genome*
14	GGKSGGNKKSDGVK	AAKSLLNKKADGVK	AAKSLLNKKSDGGVK	AAKSLLKKPDGVK
14b (in β, δ)	*no internal splice site*	AAKSLLNKKADGVK	*no internal splice site*	AAKSLLKKPDGVK
15	KKRKSSSSVQLM	*not encoded in genome*	KRKSSSSVHLM	KRKSSSSVQMM
16	*not encoded in genome*	PQTNSTKNSAAATSPKGTL PPAAL	PQSNNKNSLVSPAQEPAPLQTAM	INNKANVVTSPKENIPT PAL
16b (in γ)	*not encoded in genome*	*no internal splice site*	PQSNNKNSL VSPAQEPAPLQTAM	*no internal splice site*
17	*not encoded in genome*	EPQTTVIHNPVDGIK	EPQTTVVHNATDGIK	EPQTTVIHNPDGNK
18	(K)MESSESTNTTIEDEDTKV	ESSDSANTTIEDEDAKA	GSTESCNTTTEDEDLKGR	ESTESSNTTIEDEDVKA
18b (in γ)	*no internal splice site*	*no internal splice site*	GSTESCNTTTEDEDLKV(A)GR	*no internal splice site*
19 (β)	*not encoded in genome*	AGSTESCNTTTEDEDLK AEGPLPCPSPAPFSPLPAPS	*not encoded in genome*	*not encoded in genome*
20 (β)	*not encoded in genome*	SPRISDILNSVRRGSGTPEA EGPLSAGPPPCLSPALLGPL SSPS	*not encoded in genome*	*not encoded in genome*
21 (β)	*not encoded in genome*	SPRISDILNSVRRGSGTPEA EGPSPVGPPPCPSPTIPGPL PTPS	*not encoded in genome*	*not encoded in genome*
19 (γ)	*not encoded in genome*	*not encoded in genome*	AAPLRTGNGSSVPEGRSSRDRTAP SAGMQPQPSLCSSAM	*not encoded in genome*
19b (γ)	*not encoded in genome*	*not encoded in genome*	AAPLRTGNGSSRAAPLRTGNGSS PSAGMQPQPSLCSSAM	*not encoded in genome*

ejn_14761_f4.eps

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Gene	Citations	"Traditional" name	Linker exons											
			6	13	14	15	16	17	18	19	20	21	23	25
CaMKIIα	1,3	Original α (1), α (3)			14				18 (b)					
	6,14	α-33 (6), αB (14)			14	15			18					
CaMKIIβ	2,3	orig β		13	14 (b)		16	17	18				23	
	2,3	β'		13	14		16		18				23	
	13	β3			14		16		18			21	23	
	14,18	βe			14		16	17	18				23	
	14,18	β'e			14		16	17	18				23	
	20,22	βM		13	14			17	18	19	20	21	23	
	26	β6		13	?14			17	18				23 (b)	
	26	β7											23	
	31	βe-			14		16	17	18				23	
	31	βH		13	?14		16	17	18				23	
	31	βeH			?14		16	17	18				23	
CaMKIIγ	4,7,12	Original γ (4), γA (7), γ-a (12)		13	14	15		17	18					
	7,12,30	γB (7), γ-b (12), γ2 (30)			14		16	17	18					
	7,12,30	γC (7), γ-c (12), γ3 (30)			14			17	18					
	16	γD			14		16 (b)	17	18	19				
	16,19	γE			14		16	17	18	19				
	16	γF			14			17	18	19				
	18,25	γG (18) (diff from γG 19), γGt (25)			14		16	17	18	19 (b)				
	19,25	γG (19) (diff from γG 18), γGs (25)		13	14		16	17	18					
	18	γH (diff from γH in 21)			14		16	17	18					
	21	γH (diff from γH in 18)			14	15		17	18					
	21,30	γI (21), γ1 (30)			14	15	16	17	18					
	27	γA.B		13	14	15	16	17	18					
	29	γJ		13	14			17	18					
	29	γC-2 (noncanonical: matches γC linker, but missing 23GAFSVVRR30)			14			17	18					
	29	γG-2 (noncanonical: matches γGs linker, has different "hub" domain from intron)		13	14		16	17	18					
CaMKIIδ			?											
	5,8-12	Original δ (5), δ1 (8,9,10), δA (11) (ambig. Cterm), δ-a (12) (ambig.)	6		14		16	17	18					25
	8-11,18	δ2 (8,9,10), δC (11,18) (ambig. Cterm in both)	6		14				18					25
	8,10-12	δ3 (8) (ambig.), δ3 (10) (not detected), δB (11) (ambig.), δ-b (12) (ambig.)	6		14	15			18					25
	9,10	δ3 (9), δ5 (10)	6		14		16	17	18					
	9-12,18	δ4 (9), δ6 (10), δC (11,18) (ambig. Cterm in both), δ-c (12) (ambig.)	6		14				18					
	8-10	δ4 (8) (ambig. Cterm), δ5 (9), δ4 (10)	6		14		16		18					25
	8-10	δ4 (8) (ambig. Cterm), δ6 (9), δ8 (10)	6		14		16		18					
	10,11	δ7 (10), δB (11) (ambig. Cterm)	6		14	15			18					
	17,18	δ9 (17), δE (18) (ambig. Cterm)	6		14			17	18					25
	17,18	δ10 (17), δE (18) (ambig. Cterm)	6		14			17	18					
	21	δ11 (diff from δ11 in 23)	6		14				18					25
	23	δ11 (diff from δ11 in 21)	6		?14	15		17	18					25
	21	δ12	6		14				18					
	24	δ3.1	6		14	15	16	17	18					25?
	24	δ3.4	6		?14	15			18					25?

ejn_14761_f5.eps

This article is protected by copyright. All rights reserved