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Ca_v2.3 channel and PKC λ : new players in insulin secretion

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Insulin secretion is critically dependent on the proper function of a complex molecular network. Ca_v2.3-knockout (Ca_v2.3^{-/-}) and PKC λ -knockout (PKC λ ^{-/-}) mouse models now suggest that these 2 players, the Ca_v2.3 channel and PKC λ , are important constituents of this molecular network. Subsequent to glucose stimulation, insulin is released from the pancreatic β cell in a biphasic pattern, i.e., a rapid initial phase followed by a slower, more sustained phase. Interestingly, Ca²⁺ influx through the Ca_v2.3 channel regulates only the second phase of insulin secretion. PKC λ seems to enter the β cell nucleus and in turn modulates the expression of several genes critical for β cell secretory function. Studies by Hashimoto et al. and Jing et al. in this issue of the *JCI* set out to answer the question of why numerous isoforms of proteins with similar functions are present in the β cell. This is important, since it has been difficult to understand the modulatory and/or regulatory roles of different isoforms of proteins in defined subcellular compartments and at various times during the secretory process in both β cell physiology and pathophysiology (see the related articles beginning on pages 138 and 146).

The pancreatic β cell adequately and efficiently secretes insulin to maintain glucose homeostasis. Numerous players with distinct roles act in concert to precisely regulate the complex process of insulin secretion. The β cell relies on common mechanisms shared by other types of cells to execute exocytosis of insulin-containing granules, but also exhibits unique features. The β cell is exquisitely sensitive to glucose. Upon elevation

of the plasma glucose level, the β cell efficiently takes up glucose through glucose transporters. Thereafter, subsequent glucose metabolism results in the activation of a series of signal transduction events. A well-known paradigm demonstrates that an increase in the ATP/ADP ratio derived from glucose metabolism closes ATP-sensitive K⁺ (K_{ATP}) channels, resulting in depolarization of the plasma membrane. The membrane depolarization in turn opens Ca_v channels, mediating Ca²⁺ influx. The resultant increase in [Ca²⁺]_i triggers direct interactions between exocytotic proteins situated in the insulin-containing granule membrane and those localized in the plasma membrane. Eventually, the interaction between exocytotic

proteins initiates the fusion of insulin-containing granules with the plasma membrane, i.e., insulin exocytosis (1). There is no doubt that this K_{ATP} channel-dependent pathway plays a central role in the β cell stimulus-secretion coupling. However, abolition of this pathway does not entirely block glucose-stimulated insulin secretion. This observation has led to several significant discoveries of novel mechanisms of glucose-stimulated insulin secretion, which constitute a K_{ATP} channel-independent pathway (2). For example, application of high glucose together with activators of PKA and PKC significantly stimulates insulin secretion from the β cell even under conditions where there is neither Ca²⁺ influx through the plasma membrane nor Ca²⁺ mobilization from intracellular stores (3). These K_{ATP} channel-dependent and K_{ATP} channel-independent mechanisms operate in a highly cooperative manner, always guaranteeing adequate release of insulin to maintain normoglycemia (4).

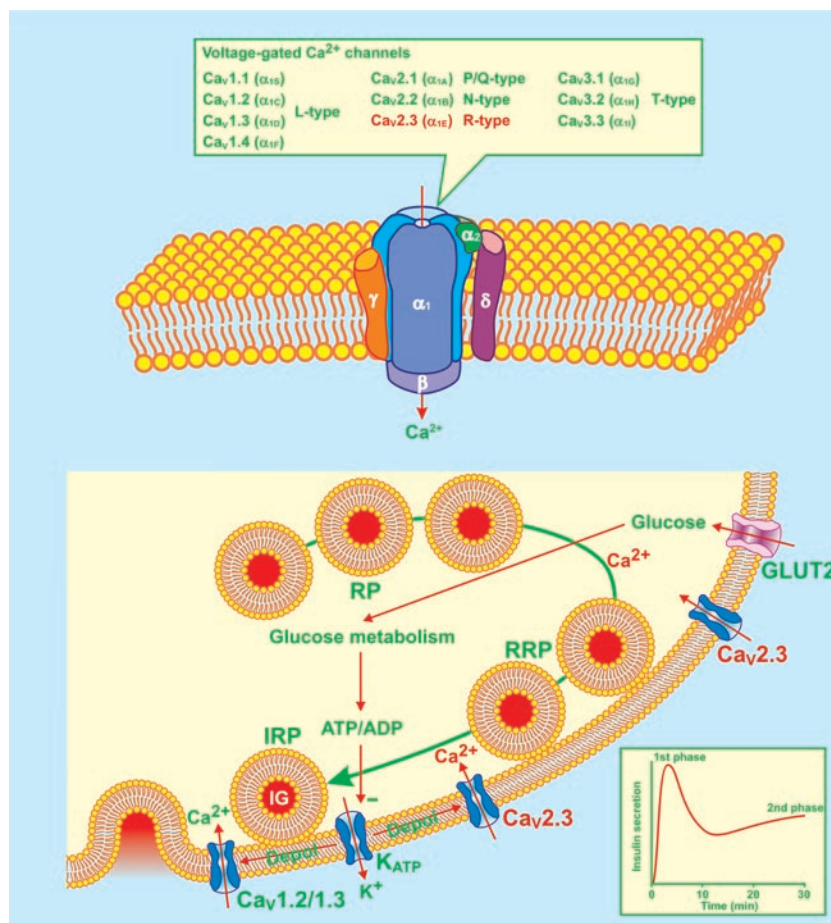
Dynamics of insulin secretion

When the β cell is exposed to an abrupt and sustained increase in the concentration of glucose, it responds with a biphasic insulin secretory pattern (Figure 1, inset). This response is characterized by a rapid initial phase of insulin release, which is maintained for about 10 minutes, followed by a nadir, and subsequently, a gradually increasing second phase, which reaches a

Nonstandard abbreviations used: Ca_v2.3^{-/-}, Ca_v2.3-knockout; DAG, diacylglycerol; K_{ATP}, ATP-sensitive K⁺; PS, phosphatidylserine.

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**Figure 1**

The functional Ca_v channel consists of pore-forming subunits Ca_vα1 and auxiliary subunits Ca_vβ, Ca_vα2/δ, and Ca_vγ. Four types of Ca_vα1 subunits, designated Ca_v1.2, Ca_v1.3, Ca_v2.1, and Ca_v2.3, conducting L-, P/Q-, and R-type Ca²⁺ currents, have been identified in the mouse β cell. Glucose-stimulated insulin secretion is characterized by a rapid first phase of insulin release for about 10 minutes, followed by a nadir, and subsequently a gradually increasing second phase reaching a plateau after 25 to 30 minutes (inset). Insulin-containing granules (IG) are functionally divided into three pools: the reserve pool (RP), the readily releasable pool (RRP), and the immediately releasable pool (IRP). The present consensus is that the K_{ATP} channel-dependent mechanisms trigger first-phase insulin secretion from the IRP by opening Ca_v1.2 and Ca_v1.3 channels. The K_{ATP} channel-independent mechanisms underlie second-phase insulin secretion by recruiting insulin-containing granules from RP and RRP to IRP. The Ca²⁺ influx through β cell Ca_v2.3 channels is now demonstrated to play a prominent role in second-phase insulin secretion. Ca_v1.2/1.3, Ca_v1.2 channels or Ca_v1.3 channels; Ca_v2.3, Ca_v2.3 channels; Depol, depolarization; GLUT2, glucose transporter 2.

plateau after a further 25 to 30 minutes. In efforts to explain biphasic insulin secretion, several models have been developed, such as compartmental, feedback, and immediate and time-dependent effect models (5–7). It has been documented that some of the K_{ATP} channel-dependent mechanisms are involved in the first phase of insulin secretion. It has been suggested that the K_{ATP} channel-independent mechanisms underlie the second phase of insulin secretion (4, 8). Moreover, it has been generally accepted that [Ca²⁺]_i regulates both first- and second-phase insulin secretion (8). The Ca_v channel-mediated Ca²⁺ influx significantly contributes to the [Ca²⁺]_i increase in the β cell (1). Although the mouse β cell carries fewer types of Ca_v channels than the β cell from other species, it is still equipped with 4 types of identified Ca_v channels, designated Ca_v1.2, Ca_v1.3, Ca_v2.1, and Ca_v2.3 (Figure 1) (1). Indeed, these Ca_v channels form a complex molecular network with other proteins, enabling the β cell to delicately secrete insulin. However, complexity makes understanding the

role of these different elements in β cell function difficult. In this issue of the *JCI*, Jing et al. (9) have successfully defined the distinct role of the Ca_v2.3 channel in second-phase insulin secretion.

Ca²⁺ influx through the Ca_v2.3 channel regulates second-phase insulin secretion

The Ca_v2.3 channel has been the most difficult subtype of Ca_v channels to evaluate the function of due to the lack of a selective blocker. The presence of the Ca_v2.3 channel in the mouse β cell was reported after the polypeptide SNX482, used as a Ca_v2.3 channel-selective blocker, was made available (10, 11). It is known that SNX482 is capable of blocking other types of Ca_v channels as well (12). Therefore, caution should be exercised when using this Ca²⁺ channel blocker to define the role of native Ca_v2.3 channels in cells containing multiple types of Ca_v channels. Jing et al. (9) combined genetic deletion of the Ca_v2.3 subunit gene and pharmacological ablation of the Ca_v2.3 channel with SNX482 in exploring the role

of the Ca_v2.3 channel in biphasic insulin secretion. This combined approach can provide assurance that the observed effects are not nonspecific or due to an insufficient selectivity of SNX482.

The work by Jing et al. (9) confirms that the Ca_v2.3 channel is present in mouse β cells and that in Ca_v2.3-knockout (Ca_v2.3^{-/-}) mice, impaired glucose homeostasis is caused by defective insulin secretion. This is in agreement with previous work from this group demonstrating that SNX482 significantly diminishes β cell Ca_v currents, causing a reduction in β cell exocytosis (11). It is of fundamental importance to determine the period when the Ca_v2.3 channel makes its contribution during biphasic insulin secretion. In this context, it is of particular interest to localize where the Ca_v2.3 channel plays its role in the complex molecular network enabling the β cell to delicately secrete insulin. It is within this framework that the study by Jing et al. sheds new light on the role of the Ca_v2.3 channel in dynamic insulin secretion. This study has been able to relate the Ca_v2.3 channel-mediated Ca²⁺

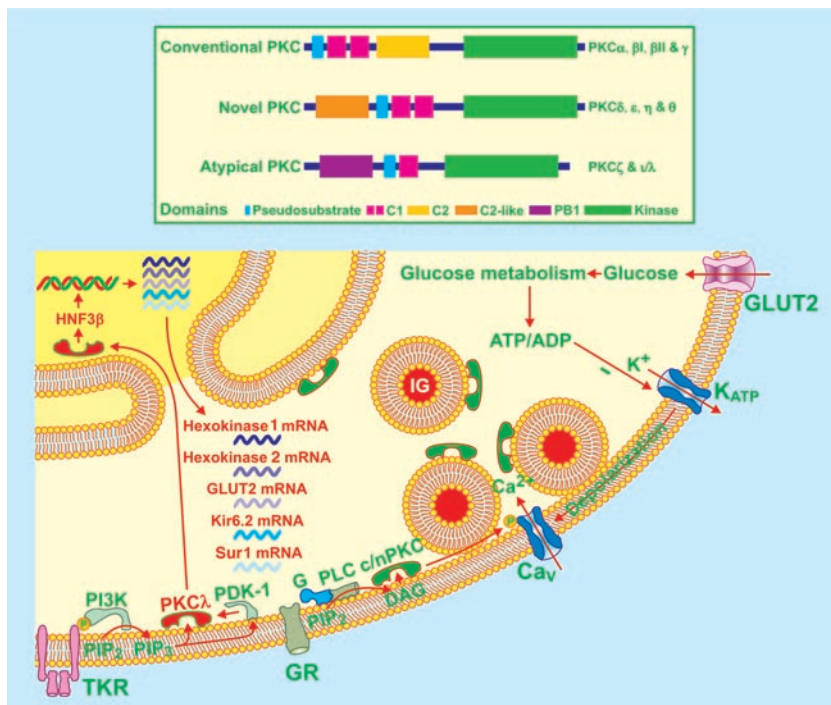


Figure 2

Ten isoforms of PKC with closely related kinase domains are classified into 3 sub-groups: conventional PKC isoforms (PKC α , PKC β I, PKC β II, and PKC γ), novel PKC isoforms (PKC δ , PKC ϵ , PKC η , and PKC θ), and atypical PKC isoforms (PKC ζ and PKC ι/λ). Multiple conventional PKC and novel PKC isoforms (c/n PKC) are involved in the regulation of insulin secretion through different targets. PKC λ in the β cell is now thought to participate in glucose-stimulated insulin secretion by modulating the expression of HNF3 β , hexokinase 1, hexokinase 2, glucose transporter 2, Kir6.2, and Sur1 subunit genes critical for β cell function. G, GTP-binding protein; GR, GTP-binding protein-coupled receptors; P, phosphoryl group; PB1, Phox and Bem 1; PDK-1, phosphatidylinositol 3-kinase-dependent kinase-1; PIP₂, phosphatidylinositol 4,5-bisphosphate; PIP₃, phosphatidylinositol 3,4,5-trisphosphate; PLC, phospholipase C; TKR, tyrosine kinase receptor.

influx to second-phase insulin secretion, where the regulatory mechanisms at the molecular level are poorly understood.

The work by Jing et al. (9) draws our attention to several important points. First, unlike the β cell Ca_v1.2 and Ca_v1.3 channels, which are tightly coupled to the exocytotic machinery (13–15), the Ca_v2.3 channel seems to be distant from the areas in the β cell where insulin-containing granules fuse with the plasma membrane and release their contents. Neither the deletion of the Ca_v2.3 subunit gene nor the pharmacological ablation of the Ca_v2.3 channel altered the exocytotic profile of insulin-containing granules from the immediately releasable pool. These manipulations also did not affect first-phase insulin secretion. This demonstrates that the Ca_v2.3 channel does not regulate the exocytotic capacity of the β cell (Figure 1).

Second, Ca²⁺ influx through the Ca_v2.3 channel mediates second-phase insulin secretion by recruiting insulin-containing granules from other pools to the immediately releasable pool. This is strongly supported by the fact that a massive decrease in insulin release occurred selectively at the second phase from either the Ca_v2.3^{-/-} islets or islets exposed to SNX482 (9). Recently, it has been hypothesized that there is 1 rate-limiting step between the 2 phases of insulin secretion (16). This key step controls the conversion of insulin-

containing granules in the readily releasable pool to the immediately releasable pool. It has been postulated that the K_{ATP} channel-independent pathway regulates this key step. A variety of signaling molecules, e.g., cyclic AMP, diacylglycerol (DAG), GTP, and ATP are involved in this pathway, resulting in second-phase insulin secretion (16). Jing et al. have added a new important player, the Ca_v2.3 channel, to this pathway. It is attractive to speculate that the Ca_v2.3 channel may segregate its Ca²⁺ influx from the Ca²⁺ influx through the Ca_v1.2 and Ca_v1.3 channels by using unknown molecular barriers or selecting different cellular locations (Figure 1).

Third, the Ca_v2.3 channel plays a role in β cell [Ca²⁺]_i oscillations. Jing et al. (9) showed that the Ca_v2.3^{-/-} β cell not only displays a reduced increase in integral [Ca²⁺]_i, but also a slower frequency of [Ca²⁺]_i oscillations following high glucose stimulation. [Ca²⁺]_i oscillations are involved in the regulation of a number of cellular processes, e.g., insulin secretion and gene expression (17, 18). Recently, we demonstrated that the fast [Ca²⁺]_i oscillations arising from Ca_v β 3 gene deletion significantly enhance insulin secretion (17). Jing et al. (9) seem not to have considered that the decreased frequency of [Ca²⁺]_i oscillations may also contribute to the decreased insulin secretion observed from Ca_v2.3^{-/-} islets. We believe that both the amount of [Ca²⁺]_i and the [Ca²⁺]_i oscilla-

tion frequency affect insulin secretion. This is strongly supported by the data obtained by Jing et al., which showed a 20% decrease in integral [Ca²⁺]_i, a 30% decrease in [Ca²⁺]_i oscillation frequency, and a 50% decrease in insulin secretion from Ca_v2.3^{-/-} islets. Additionally, it is of particular interest that Ca_v2.3^{-/-} islets contain less-differentiated islet cells. This phenomenon may also correlate with the reduced frequency of [Ca²⁺]_i oscillations in Ca_v2.3^{-/-} islets. It is reasonable to assume that a proper frequency of [Ca²⁺]_i oscillations drives the expression of some genes critical for β cell differentiation since [Ca²⁺]_i oscillations have been shown to regulate gene expression (18). The adult pancreas maintains adaptive β cell growth via differentiation from pancreatic ductal cells and replication from already existing β cells (19). In vivo β cell regeneration has been demonstrated in human subjects (19). Understanding how the Ca_v2.3 channel-mediated [Ca²⁺]_i oscillations contribute to β cell regeneration is therefore of interest. It may provide new knowledge regarding β cell growth and lead to the development of novel therapeutic approaches to diabetes that selectively manipulate Ca_v2.3 channel expression.

It should be pointed out that the reduction in integral [Ca²⁺]_i and [Ca²⁺]_i oscillation frequency in Ca_v2.3^{-/-} islets observed by Jing et al. (9) might also decrease Ca²⁺-dependent adenylyl cyclase and phospholipase C activities (20, 21). The resultant decreases in



the production of cAMP and DAG may in turn attenuate second-phase insulin secretion (16). It would be important to explore these possibilities in future studies.

PKC isoforms in β cells

Ten isoforms of PKC with closely related kinase domains are classified into 3 subgroups in terms of the differences in their function and structure. The conventional PKC isoforms (PKC α , PKC β I, PKC β II, and PKC γ) are Ca²⁺ dependent and sensitive to both DAG and phosphatidylserine (PS). The novel PKC isoforms (PKC δ , PKC ϵ , PKC η , and PKC θ) are sensitive to both DAG and PS, but Ca²⁺ independent. The atypical PKC isoforms (PKC ζ and PKC ι/λ) are Ca²⁺ independent and DAG insensitive, but sensitive to PS (Figure 2) (22). Two additional isoforms of PKC, PKC μ and PKC ν , have also been cloned (22). The β cell is equipped with multiple PKC isoforms, which are involved in the regulation of insulin secretion via multiple targets (23–26). PKC λ is equipped with nuclear import and export signals. Therefore, activated PKC λ can quickly translocate to the nucleus (Figure 2) (27). This isoform has also been visualized in the β cell. In vitro experiments suggest that atypical PKC is involved in both glucose-stimulated insulin secretion and glucose-induced gene transcription (28, 29). However, the different isoforms of atypical PKC cannot be differentiated by available pharmacological reagents even in in vitro experiments (30). Neither the in vitro nor the in vivo function of PKC λ in the β cell is known.

PKC λ modulates the expression of several genes critical for β cell secretory function

In this issue of the *JCI*, Hashimoto et al. (31) report that they have created β PKC $\lambda^{-/-}$ mice. Using these knockout mice, they show for the first time to our knowledge that, in vivo, PKC λ regulates glucose-stimulated insulin secretion by maintaining proper expression of some genes critical for β cell function. The work by Hashimoto et al. (31) demonstrates that the β PKC $\lambda^{-/-}$ mice, without compensatory increase of β cell PKC ζ , another atypical PKC isoform, exhibit glucose intolerance with decreased glucose-stimulated insulin secretion without significant insulin resistance. The isolated β PKC $\lambda^{-/-}$ islets are characterized by an elevation in basal insulin secretion and a reduction in glucose-stimulated insulin secretion resembling the profile of insulin secretion from type 2 diabetic islets (7).

The question that immediately arises is how deletion of one single gene for PKC λ in the β cell causes complicated changes in insulin secretion, i.e., more basal insulin release and less glucose-stimulated insulin secretion. To address this question, Hashimoto et al. (31) examined possible changes in the function, morphology, and gene expression of the β PKC $\lambda^{-/-}$ islets with regard to insulin secretion. Interestingly, the β PKC $\lambda^{-/-}$ islets showed complicated alterations in expressions of several genes critical for β cell function, including glucose transporter 2, HNF3 β , and hexokinase 1 and 2 as well as Kir6.2 and Sur1 subunits of K_{ATP} channels. Increases in hexokinase 1 and 2 expression may explain elevated basal insulin secretion. Decreases in expressions of glucose transporter 2, HNF3 β , Kir6.2, and Sur1 subunits may consequently account for impaired glucose-stimulated insulin secretion (Figure 2). Importantly, introduction of the PKC λ gene in β PKC $\lambda^{-/-}$ islets normalized hexokinase 1, HNF3 β , and Kir6.2 subunit expression and in turn reversed elevated basal insulin release as well as blunted glucose-stimulated insulin secretion to normal. The increased basal insulin release and blunted insulin response to high glucose also became normal following restoration of HNF3 β expression. It is clear that PKC λ plays a crucial role in controlling the expression of multiple genes critical for β cell secretory function. Therefore, disturbed expression of the PKC λ gene may be involved in the development of the polygenetic disease type 2 diabetes. This is in line with the findings by Hashimoto et al. (31), namely that β PKC λ knockout caused a type 2 diabetes-like phenotype including a higher basal and lower glucose-stimulated insulin release as well as glucose intolerance. This may also suggest that the gene encoding PKC λ could serve as a potential therapeutic target.

It should be kept in mind that sometimes protein function is not directly proportional to protein abundance. For example, a very small percentage of K_{ATP} channels are kept open in the normal β cell at basal glucose levels (32). It is therefore crucial to evaluate the activity of hexokinases, glucose transporter 2, and K_{ATP} channels in PKC $\lambda^{-/-}$ β cells to clarify to what extent changes in the amounts of the actual mRNAs and proteins indeed correlate with alterations in their function. Furthermore, type 2 diabetes is characterized by a blunted first phase and a right-shifted second phase of insulin secretion. Therefore, it is of particular interest to further examine how β PKC λ knockout affects the kinetics of biphasic insulin secretion.

In general, the findings of these 2 studies are important since they reveal specific functions for defined protein isoforms in pancreatic β cell signal-transduction. This field of research will continue to be a significant challenge for us when trying to understand the roles of different protein isoforms in the dynamic and complex processes of both physiological and pathophysiological insulin secretion. This is of particular interest when trying to define novel glucose-dependent drugable targets in the treatment of type 2 diabetes. In terms of clinical relevance, the roles of Ca_v2.3 and PKC λ need to be verified in human β cell signal transduction.

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A new mechanism of BRAF activation in human thyroid papillary carcinomas

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In this issue of the *JCI*, Ciampi et al. report the identification of a novel oncogene in patients affected by radiation-associated thyroid papillary carcinomas (see the related article beginning on page 94). This oncogene derives from a paracentric inversion of the long arm of chromosome 7, which results in an in-frame fusion of the N-terminus of the *A-kinase anchor protein 9* (*AKAP9*) gene with the C-terminal catalytic domain (exons 9–18) of the serine-threonine kinase *BRAF*. The resulting *AKAP9-BRAF* fusion protein shows constitutive kinase activity, and it is able to transmit mitogenic signals to the MAPK pathways and to promote malignant transformation of NIH3T3 cells.

The Chernobyl nuclear power plant accident in 1986 caused severe radioiodide contamination of several areas in Belarus, Ukraine, and western Russia, leading to high radioactive exposure of the thyroid gland among the general population, including children. Beginning in 1992, a sharp increase in the incidence of childhood thyroid tumors, predominantly papillary thyroid carcinomas (PTCs), was

reported. The molecular analysis of these tumors has provided a unique opportunity to study the mechanisms of radiation-dependent carcinogenesis in humans (1).

In this issue of the *JCI*, Ciampi et al. (2) report the identification of a novel oncogene in PTCs that developed in irradiated patients after a short latency period. This oncogene derives from the in-frame fusion of the first 8 exons of the *A-kinase anchor protein 9* (*AKAP9*) gene with the C-terminal-encoding region (exons 9–18) of the proto-oncogene *BRAF*. *AKAP9-BRAF* fusion results from a paracentric inversion [inv (7)(q21–22q34)] of the long arm of chromosome 7 (Figure 1). *BRAF* is a serine-threonine kinase involved in the transmis-

sion of signals from membrane receptors and RAS small GTPases to MAPK (Figure 2). This pathway transduces mitogenic signals in response to the activation of tyrosine kinase receptors. The *AKAP9-BRAF* fusion event results in the loss of 2 *BRAF* regulatory domains, CR1 and CR2 (Figure 2), which exert autoinhibitory effects on the kinase activity of *BRAF*; CR1 includes the RAS-GTP binding domain (3). Accordingly, the *AKAP9-BRAF* recombination and the loss of CR1 results in a RAS-independent gain-of-function of *BRAF* that is able to induce transformation of NIH3T3 cells that become tumorigenic after injection into athymic mice.

Oncogene activation in human PTCs

The *AKAP9-BRAF* fusion event reported by Ciampi and coworkers (2) represents an additional example of oncogene formation due to chromosomal rearrangements in human PTCs. Other examples include *RET/PTC* and *TRK-T* oncogenes. *RET/PTC*, present in about 40% of human PTCs, are chimeric genes generated by the fusion of the C-terminal catalytic domain of the *RET*

Nonstandard abbreviations used: *AKAP9*, *A-kinase anchor protein 9*; FA, follicular adenoma; FTC, follicular thyroid carcinoma; PTC, papillary thyroid carcinoma.

Conflict of interest: The authors have declared that no conflict of interest exists.

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