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Heterogeneity of Calcium Channel/cAMP-Dependent Transcriptional Activation

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Abstract

The major function of the voltage-gated calcium channels is to provide the Ca^{2+} flux into the cell. L-type voltage-gated calcium channels (Ca_v1) serve as voltage sensors that couple membrane depolarization to many intracellular processes. Electrical activity in excitable cells affects gene expression through signaling pathways involved in the excitation-transcription (E-T) coupling. E-T coupling starts with activation of the Ca_v1 channel and results in initiation of the cAMP-response element binding protein (CREB)-dependent transcription. In this review we discuss the new quantitative approaches to measuring E-T signaling events. We describe the use of wavelet transform to detect heterogeneity of transcriptional activation in nuclei. Furthermore, we discuss the properties of discovered microdomains of nuclear signaling associated with the E-T coupling and the basis of the frequency-dependent transcriptional regulation.

Keywords

Image analysis; microdomain; signaling; transcription; voltage-gated calcium channels; wavelet

VOLTAGE-GATED Ca^{2+} CHANNEL SERVES AS THE VOLTAGE SENSOR FOR INTRACELLULAR PROCESSES

Harold Reuter's discovery of calcium current in the heart showed that Ca^{2+} enters into the heart cells through the Ca^{2+} channel [1]. Intensive research in this field has revealed that Ca^{2+} channel is not only the molecular machinery allowing Ca^{2+} to enter the cells, but it is also a voltage sensor that can couple changes of transmembrane voltage to the intracellular processes. Changes of transmembrane potential in neuronal and muscle cells induce a variety of intracellular events. The major function in muscle cells is the contraction. Contraction in muscle cells is induced and regulated by depolarization of the plasma membrane. The coupling between these events was named the excitation-contraction coupling (E-C). It was well established that the role of voltage sensor for E-C coupling is served by L-type voltage gated calcium channel (Ca_v1) [2, 3]. The pore forming subunit (α) of skeletal isoform $\text{Ca}_v1.1$ serves as a voltage sensor for initiation of skeletal muscle

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CONFLICT OF INTEREST

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contraction without requiring Ca^{2+} flux through the pore subunit. E-C coupling in skeletal muscle is induced by direct interaction between α subunit of $\text{Ca}_v1.1$ channel and the ryanodine receptor (RyR) intracellular calcium release channel. Voltage-induced conformational changes of the α subunit $\text{Ca}_v1.1$ channel during depolarization of skeletal muscle results in opening of sarcoplasmic RyR channel and massive calcium release from sarcoplasmic reticulum due to physical interaction between $\text{Ca}_v1.1$ channel and RyR [2]. There is another isoform of L-type voltage gated calcium ($\text{Ca}_v1.2$) serving as a voltage sensor in the heart. The pore forming α subunits of $\text{Ca}_v1.2$ channel are widely expressed in the heart cells [4–6]. These subunits have no physical contact with the RyR channel of the sarcoplasmic reticulum. EC coupling in heart muscle is mediated by calcium flux through the pore forming a subunit of $\text{Ca}_v1.2$ and consequent Ca-induced Ca release through the RyR channels. The coupling of L-type Ca_v1 channels activity with the secretion of hormones from endocrine cells [7] or neurotransmitter release from auditory hair cells [8] and photoreceptors [9] is the excitation-secretion coupling. The major function of neuronal cells is to transmit and process information. These processes require constant dynamic changes of electrical activity. The L-type voltage gated calcium channel accounts for 80% of voltage gated Ca^{2+} channels in the central nervous system. Ca_v1 channels are mainly localized in postsynaptic structures and play an important role in the reception of incoming signals [10]. It has been discovered more than a decade ago that Ca^{2+} entering via Ca_v1 channels has a unique ability to convert these incoming signals in transcriptional regulation inside nuclei of neurons and muscle cells [11, 12].

Neuronal activity regulates gene transcription by activating plasma membrane receptors or triggering calcium influx through the voltage-gated and ligand-gated calcium channels. Several hundred neuronal activity-regulated genes have been identified [13]. Complex membrane-initiated signaling networks including glutamate receptor, brain-derived neurotrophic factor, and extracellular signal-regulated kinase activated pathways regulate transcription in activity-dependent manner [14]. Electrical activity of excitable cells affects gene expression. The functional link between the changes of plasma membrane voltage and corresponding changes in transcriptional regulation of gene expression in nuclei was named as the excitation-transcription (E-T) coupling [11, 12]. E-T coupling is one of the possible signaling pathways of activity-dependent gene expression control, but E-T coupling provides the strongest correlation (gain) between membrane depolarization and transcriptional activation. One of the most dominant signaling pathways regulating transcriptional activity in neuronal and muscle cells is the cAMP-response-element-binding protein (CREB) dependent pathway. This pathway is controlled by the activity of second messengers - cAMP and calcium [15, 16]. E-T coupling is critical for neuronal plasticity [17] and development of compensatory hypertrophy in cardiac muscle [18].

The unique role of the L-type voltage-gated Ca^{2+} channel ($\text{Ca}_v1.2$) as the voltage sensor in the transmission of the Ca^{2+} signal to the nucleus in E-T coupling in neurons and muscle cells has been established [3]. In recent years, a few quantitative experimental approaches were developed to understand how the nuclei receive information from the plasma membrane.

QUANTITATIVE METHOD TO STUDY EXCITATION-TRANSCRIPTION COUPLING BY INTEGRATION OF SIGNAL

The sensitivity of signaling to the changes of transmembrane voltage determines the effectiveness of excitation–response coupling. To measure the effectiveness of E-T coupling, the changes of transmembrane potential or changes in $\text{Ca}_v1.2$ channel activity are compared and correlated with the changes of the induced integral response in phosphorylation of CREB in the whole nuclei as a measure of activation of CREB-dependent transcription (CDT).

The control of the plasma membrane voltage can be achieved in two ways: either by changing the extracellular concentration of K^+ or by changing the voltage directly in the perforated patch clamp experiments (the size of the hole is important for preservation of the intracellular environment). Both methods have advantages and disadvantages: by applying a patch clamp protocol one can dynamically control voltage and Ca_v1 channels activity; by changing the K^+ solution one may have opportunity to obtain data from a large number of cells simultaneously.

Activation of $\text{Ca}_v1.2$ channel initiates CDT. PKA-mediated phosphorylation of CREB on Ser133 is essential for activation of a transcriptional response to second messenger cAMP [16]. Therefore phosphorylation of CREB inside the nuclei can be used as an integrated parameter for measuring the strength of transcriptional signaling in CDT activation. In this experimental approach, cells were depolarized with different concentrations of K^+ and then fixed [18]. The fixed cells were sequentially incubated with a primary antibody to CREB Ser133 and then with a secondary ALEXA-488 antibody.

The level of CDT activation corresponded to the intensity of the fluorescent signal over the whole nuclear region. This signal was compared to the changes of transmembrane potential. The initial slope of the curve of the level of CREB phosphorylation over time of cell depolarization was estimated. This parameter (the slope) represented the “CREB signaling strength”. This signaling strength was steeply dependent on depolarization. Comparison of measured excitation-response coupling in different types of cell showed that voltage-dependence of E-T coupling was different from E-C (in cardiac myocytes or skeletal muscle) and E-S coupling (in neuroendocrine cells) [18, 19].

This integrated approach was implemented under the assumption that the signaling process in the nuclei is homogeneous in space and time. The limitations of this method may result in underestimation of important local signaling events either transient in nature or confined in space. In reality a mammalian cell nucleus is structurally and functionally complex and contains morphologically distinct chromatin domains and numerous protein subcompartments with chromosomes occupying discrete, nonrandom positions inside a nucleus [20]. The gene expression is organized in spatially heterogeneous subnuclear domains [21]. The spatial organization of these nuclear compartments defines their role in gene expression and cell regulation [22]. The dynamic organization of the transcriptional compartments and the position of chromosomes provide the basis of the dynamic complexity of the E-T coupling of actively transcribed nuclear microdomains [23].

Because of this spatio-temporal complexity of nuclear organization, by using an integral approach one can easily overlook the transient and local transcriptional activity. Therefore, identification of the spatio-temporal activity of transcriptional compartments organized in signaling microdomains is very important in investigation of the transcriptional signaling [24].

QUANTITATIVE SPATIO-TEMPORAL WAVELET ANALYSIS OF MICRODOMAIN ORGANIZATION OF EXCITATION – TRANSCRIPTION COUPLING NUCLEAR SIGNALING

We performed FRET microscopy imaging of CDT activation in live cells expressing recombinant (COS1 or HEK293) or endogenous (cardiac myocytes) $\text{Ca}_v1.2$ combined with a perforated patch clamp [24, 25]. These methods allowed effective control over activation of CDT by stimulation of the inward Ca^{2+} current through $\text{Ca}_v1.2$ channels. Intranuclear CDT signaling measured as a FRET signal of endogenously expressed interacting domains of CREB and CREB binding protein (CBP) fused to EYFP and ECFP, respectively [24].

In cell microscopy imaging of transcriptional activation, each pixel of the recorded nuclear image contains information regarding activity of signaling events. In order to measure the heterogeneous signaling events we have to identify signaling domains, i.e., pixels or groups of pixels representing specific activity. The appropriate statistical tools should be used in the estimation of the degree of significance of changes in signaling events over background noise. First, we have to identify groups of pixels with visually strong changes in the intensity of FRET signal over background (potential signaling intranuclear domains), and second, we have to apply a statistical test to estimate the significance of these changes (real signaling intranuclear domains) [26]. To reach this important goal, we proposed to apply wavelet transform to the analysis of E-T coupling in the live cell (Fig. 1A) [26, 27]. To correlate signaling events inside the nucleus with the activity of $\text{Ca}_v1.2$ in the plasma membrane, we simultaneously recorded two parameters: excitability of the plasma membrane/activation of $\text{Ca}_v1.2$ channels and CDT activation measured as a FRET signal [24, 25].

For identification of possible microdomains of the CREB-dependent transcriptional activation we employed the wavelet transformation of 2D images. This transformation allows us to estimate global and local heterogeneity in the entire 2D image. Continuous wavelet transform (CWT) is an extension of Fourier analysis but colocalizes in both the space and frequency domains to permit assessment of changes in spatial frequency content of obtained 2D images. Thus, this mathematical technique allows us to analyze over several different frequencies across the entire signal [26, 27]. To do this, we compare how a similar signal (spatial distribution intensity in number of pixels) compares to a wavelet function across the entire image. For each local region (group of pixels) we get a measure of how well the wavelet correlates to the signal it is compared to, called a scale coefficient. We then shift the wavelet to the next region in the image and measure another coefficient, and so on until we cover the whole signaling event with the chosen wavelet. Then, we change the frequency of the wavelet by stretching or contracting it, an operation known as scaling the wavelet, and compare the signal to the newly scaled wavelet.

By repeating this over a number of different scales (spatial frequencies of the wavelet), we can find the regions that show a very strong signal resembling the wavelet function. The heterogeneous nature of wavelet function indicated that we observe heterogeneity in these regions (group of pixels) in the distribution of signal intensity. Using this mathematical technique permitting simultaneously global and local analysis of the signaling event [25], we can find out whether in a given time series signal X there are drastic changes in frequency and amplitude, and how the two time series are related in frequency and phase. To implement this powerful analytical method to the study of transcriptional activation, we developed continuous 2D wavelet transform as a deconvolution algorithm for FRET microscopy image analysis of CREB-dependent transcriptional activation [26]. This statistical tool is particularly useful for the identification of heterogeneity in a signal, as it can easily find where the pattern (i.e., frequency) of signal changes in time and space. We used this method to identify localized regions of increased FRET intensity of the CREB transcriptional probes and observe their dynamic changes within the nucleus. The term microdomain was used because of the submicrometer size of these CDT signaling domains (usually these domains are a few pixels large, pixel size ~ 250 nm) and to avoid confusion with nanodomains (scale of a single molecule, from a few to 100 nm). The spatial resolution of wavelet analysis is limited by optical resolution: ~ 250 nm for regular microscopy. The use of super-resolution microscopy may be beneficial to increase the optical resolution and power of the wavelet analysis. The detailed protocols and description of wavelet analysis of FRET microscopy images were described in a series of our papers [25–28].

The FRET images were analyzed as coefficient matrices. Normalized differences in matrices of the wavelet coefficients were used to initially identify the potential microdomains (Fig. 1B). All potential microdomains were subject of the statistical comparison (Fig. 1B,C). Spatio-temporal pattern of signaling activity was compared in already identified signaling domains. Our quantitative approach revealed the existence of a significant heterogeneity of CDT activity. By using wavelet transform analysis we were able to identify the transient and stable discrete CDT signaling intranuclear domains.

The heart cells and some neurons exhibited periodic electrical activity. Periodic activation of Ca_v1 channels may lead to frequency modulation of the CDT activation. To measure the degree of synchronicity coherence of the periodic activation of $Ca_v1.2$ channels and initiation of transcriptional activation in the identified spatially different segregated nuclear microdomains, we applied time-series statistical analysis. Wavelet transform coherence (WTC) was used to analyze the local correlation between two simultaneously recorded processes: membrane excitability (contractility) and nuclear transcriptional activity (FRET signals). 1D continuous wavelet transform analysis was applied to the time series of the plasma membrane excitability and nuclear CDT activity. By using WTC we observed a significant coherence between excitability of the plasma membrane and intranuclear transcriptional activation [25].

We have presented two different quantitative approaches in studying E-T coupling. The integral approach is based on the recording of the total fluorescent signal from the nuclei. This quantitative method was successfully used in the estimation of the extent and strength

of coupling between the changes in transmembrane potential (excitation) and the CREB phosphorylation inside the nuclei (transcription). The second approach is based on the quantitative microdomain analysis that was developed in our laboratory. We performed spatial and time-series analysis with the wavelet tools on FRET reporter signals from nuclei. This wavelet-based method was aimed at detecting spatially-segregated signaling microdomains of transcriptional activity. To estimate the level of synchronicity and coherence between the excitability of the plasma membrane and CDT activation in nuclear microdomains we applied the WTC analysis. This wavelet-based quantitative approach allowed us to analyze the heterogeneity of CDT signaling during the E-T coupling.

EXCITATION-TRANSCRIPTION COUPLING AND HETEROGENEITY OF NUCLEAR SIGNALING

Activity of the L-type $\text{Ca}_v1.2$ calcium channels during membrane depolarization induces transient increase in the intracellular free calcium concentration, which in part is involved in the regulation of the CDT activity. However, other types of voltage-gated calcium channels (*e.g.*, the N and P/Q type Ca_v2 channels) also provided a strong increase in the intracellular free calcium, but for some reason these voltage-gated calcium channels are much less effective in the E-T coupling [30]. What are the reasons for the superiority of the $\text{Ca}_v1.2$ channels over Ca_v2 channels in the E-T coupling? First, $\text{Ca}_v1.2$ channels activate at more negative potentials and the probability of opening for $\text{Ca}_v1.2$ channels is higher at a relatively moderate depolarization [30]. This gating advantage at low voltages provides a lower threshold for maximal signal strength response in the E-T coupling. Second, specific organization of $\text{Ca}_v1.2$ channels in multimolecular signaling complexes with CaMKII results in 10 times more efficient signaling to nuclei. Ca^{2+} entering through Ca_v2 channels must act at a considerably greater distance from the site of Ca^{2+} entry ($\sim 1 \mu\text{m}$), while $\text{Ca}_v1.2$ channels signal in the vicinity of the pore by activating CaMKII. This specificity of $\text{Ca}_v1.2$ channels multimolecular signaling complexes explains why the local rise of intracellular Ca^{2+} in the vicinity of the $\text{Ca}_v1.2$ channel induces effective E-T coupling events.

Our FRET experiments under patch clamp conditions in the live COS1 cells expressing fully functional endogenous $\text{Ca}_v1.2$ containing ECFP/EYFP-labeled α_{1C} N- and Ctermini, showed large voltage-gated rearrangements of this C-terminal tail [24]. Inhibition of the mobility of the C-tail by its anchoring in the membrane did not decrease the calcium current. In fact, the total Ca^{2+} influx was much larger due to inhibition of the channel inactivation. However, the anchoring of the α_{1C} subunit C-terminal tail completely inhibited activation of the CREB-dependent transcription even with the CaMKII consistently present near the conducting $\text{Ca}_v1.2$ pore. The complete restoration of the voltage-gated mobility and transcriptional activation was successfully achieved after release of the tail from the inner leaflet of the plasma membrane [24]. Thus, neither the large inward Ca^{2+} current nor the massive intracellular Ca^{2+} release, induced by the activation of G-protein coupled receptors led to the CREB-dependent transcription activation. Conformational change of the $\text{Ca}_v1.2$ channel provided the voltage-gated shuttling of CaM as Ca^{2+} carrier between the pore and the target by the mobile α_{1C} subunit C-tail, which underlies activation and inactivation of

the $\text{Ca}_v1.2$ channel [31]. This voltage-dependent conformational rearrangement of the $\text{Ca}_v1.2$ C-tail was important for the CREB-dependent transcriptional activation. Recent data [12, 18, 30, 31] showed that calmodulin and CaMKII are crucial signaling molecules in E-T coupling. CaMKII acting in the vicinity of the $\text{Ca}_v1.2$ channel transmits and amplifies the Ca^{2+} signal to nuclei to induce the CDT activation by responding to the frequency of the $\text{Ca}_v1.2$ openings rather than to the net Ca^{2+} flux [30, 32].

It is known that proteolytic cleavage of full-length $\text{Ca}_v1.2$ channel generates a distal C-terminus fragment that may function as a transcriptional regulator in the nucleus [33, 34]. Proteolysis of the C-terminus is a one-time event in relatively long life of the $\text{Ca}_v1.2$ channel protein. Therefore, the cleavage of the C-terminus of $\text{Ca}_v1.2$ channel can't continually transmit signals from the plasma membrane to the nucleus in the process of E-T coupling. The remaining truncated $\text{Ca}_v1.2$ channel continues to function as a voltage sensor and Ca^{2+} channel without significant problems due to the fact that the major binding sites, including CaM remain intact on the proximal C-terminus.

Is CaMKII involvement in the CREB-dependent transcriptional activation obsolete? We analyzed spatio-temporal organization of the CDT activity in a more detailed way, then simply averaged the FRET signal inside nuclei. Our study based on wavelet analysis showed [25] that the four major types of CDT signaling microdomains underlie the architecture of CDT signaling in COS1 cells expressing recombinant $\text{Ca}_v1.2$ calcium channels, as well as in native cardiac myocytes. The majority of signaling domains responded to both cAMP external application and the $\text{Ca}_v1.2$ channel stimulation, showing sustained activity. These domains constitute major changes in discrete FRET signals that essentially reflect the average nuclear FRET changes, measured by the conventional integral approach. A number of stable CDT domains activated only by cAMP was presented by significantly smaller fraction of cardiac myocytes nuclear microdomains (~ 10% of all CDTdomains). There were significant numbers of stable CDT domains activated only by Ca^{2+} flux (30% in cardiac myocytes and 15% in COS1 cells). Intensity of FRET signal in transient CDT microdomains was characterized by a significant initial increase in response to Ca^{2+} flux or cAMP stimulation that, however, returned to the control level during the stimulation. These transient CDT microdomains constituted approximately a quarter of all CDT nuclear domains in cardiac myocytes. We did not observe such transient domains sensitive to one of the stimuli. The CaMKII inhibitor KN93 completely abolished cAMP-induced CDT signaling in cardiac myocytes. However, about 30% of the basal CDT activity in spontaneously-contracted cardiac myocytes was dependent from the $\text{Ca}_v1.2$ channel, but not affected by the CaMKII inhibitor, and, therefore, does not depend on the CaMKII phosphorylation. Therefore, in cardiac myocytes, the CREB-dependent E-T coupling is partially executed through the CaMKII independent pathways that have not been observed in neurons. The similarity of the CDT spatiotemporal organization in cardiac myocytes and recombinant COS1 cells suggests that the basal structure of CDT signaling is inherited in a wide range of different cells.

In spontaneously contracting cardiac myocytes we were able to detect transient microdomains showing periodic CDT activity. Cells from different tissues exhibit periodic oscillations in gene expression [35]. The CREB level in cardiac myocytes was dependent

upon pace [36, 37]. The heart beats periodically. This periodic excitability of cardiac myocytes is known to result from the periodic Ca^{2+} current activation of $\text{Ca}_v1.2$ channels. The different rhythms of the heart beat are accompanied by different rhythms of Ca^{2+} influx through $\text{Ca}_v1.2$ channels and result in frequency modulation of transcriptional signaling [38]. Frequency-dependent CDT signaling events in transient nuclear microdomains in spontaneously contracting rat neonatal cardiac myocytes were synchronized with contractions and were sensitive to the DHP Ca_v1 channel blocker [25]. Ca^{2+} influx through the $\text{Ca}_v1.2$ channel resulted in contraction of cardiac myocytes. By comparison with contraction activity and average FRET intensity of a single CDT transient microdomain in time, we were able to detect a linkage between the Ca^{2+} influx through the $\text{Ca}_v1.2$ channel and the transcription activity (E-T coupling) on both small and large time scales. Indeed, WTC analysis confirmed that the E-T coupling of spontaneously contracting cardiac myocytes (contraction and transcription activity) showed evidence of a phase-locked relationship [25].

To visualize a spatial organization of nuclear CDT domains, we combined confocal microscopy with a 3D wavelet and cross-correlation analyses. We performed a 3D FRET recording by z-scanning before and during cAMP application. Each FRET image of corresponding z-slice was subject to a 2D-CWT analysis. The resultant FRET image containing only nuclear CDT signaling microdomains was used for a 3D reconstruction (Fig. 1D). This 3D reconstruction revealed a spatial intranuclear organization of cAMP-dependent microdomains of CREB and CBP interaction. Some of these domains are on the periphery of the nucleus, some are localized closer to the center. The difference in nuclear location of these microdomains may be related to the difference in their functional activity. It is known [39] that gene-rich and transcriptionally more active chromosomes tend to be located in the nuclear interior, whereas less active chromosomes are closer to the nuclear periphery. The loss of interaction with the nuclear periphery may prime the genes for transcriptional upregulation. As expected, there was no CDT signaling observed in the nucleolar region, confirming specificity of wavelet-based analysis.

Transient interactions of CREB and CBP (transient microdomains) may play the most important part in the pacing (frequency)-dependent regulation of transcriptional activity in the heart [38]. Transient nature CREB and CBP interactions are known to be associated with the stimulation of CREB activity by phosphorylation of serine-133, as well as by the activity of serine/threonine protein phosphatase [40] with additional contribution of phosphorylation of serine-142 and 143 of CREB [41]. The dynamics of phosphorylation/dephosphorylation of the CREB regulatory sites may provide a negative feedback that is necessary for the periodicity of the CREB and CBP interaction in transient microdomains underlying frequency modulation of the E-T coupling signaling in the heart cells. The wavelet transform analysis of heterogeneity of nuclear signaling events may help in our understanding of the E-T coupling.

CONCLUSIONS

E-T coupling is the regulation of gene expression by electrical activity in plasma membrane excitable cells. Ca^{2+} and cAMP are one of the major regulators of the initiation of

transcription in neurons and cardiac myocytes. CDT signaling is the major pathway of the E-T coupling. Voltage-gated $\text{Ca}_v1.2$ channels have a privileged role as voltage sensors in the initiation of the E-T coupling process. Recent progress in the E-T coupling investigation has been achieved by using different quantitative methods. The voltage-induced conformational change of the C-terminal tail of $\text{Ca}_v1.2 \alpha_1$ subunit, CaM, CaMKII, and local calcium signaling - all are important factors in transmitting activation signals to the nuclei.

Intranuclear transcriptional signaling is organized in spatially-segregated microdomains. The majority of the CDT microdomains in the nuclei of heart cells are stable, and respond to both increase of intracellular cAMP and the Ca^{2+} influx through $\text{Ca}_v1.2$ channel. The CDT domains showing transient activity and exhibiting periodic behavior may serve as the frequency modulation of CREB-dependent transcriptional signaling in the heart. These transient CDT domains may provide the basis for frequency-dependent regulation of transcriptional activity in the heart. About 30% of basal CREB/CBP interactions in heart cells depend on $\text{Ca}_v1.2$ channel activity but, unlike in neurons, are not affected by the CaMKII inhibition. It would be important to compare the fine intranuclear architecture and dynamics of the CREB-dependent transcriptional signaling in cardiomyocytes and neurons by using a quantitative wavelet-based analysis of intranuclear domains.

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Biography

Evgeny Kobrinisky

LIST OF ABBREVIATIONS

CaM	Calmodulin
CaMKII	Ca^{2+} - Calmodulin-Dependent Protein Kinase II
cAMP	Cyclic Adenosine Monophosphate
Ca_v1	L-Type Voltage-Gated Calcium Channel
CBP	CREB Binding Protein
CDT	CREB-Dependent Transcription
CREB	cAMP Response Element Binding Protein
CWT	Continuous Wavelet Transform

E-C	Excitation-Contraction Coupling
ECFP	Enhanced Cyan Fluorescent Protein
E-T	Excitation-Transcription Coupling
EYFP	Enhanced Yellow Fluorescent Protein
FRET	Fluorescence Resonance Energy Transfer
PKA	Protein Kinase A
RyR	Ryanodine Receptor

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Fig. (1).

Identification of micro- and nanodomains of the CREB-dependent transcriptional activation in nuclei of COS1 cells. **(A)** 3D representation of wavelet function (*mexican hat*) used for intranuclear microdomain identification. **(B)** Images of FRET signal representing interaction of the CREB and CREB-binding protein, expressed in COS1 cells recorded before (control) and 3 or 5 min after forskolin (10 μ M) application. Overlay of microdomains identified by 2D-CWT analysis, and those revealed as statistically significant single pixels in pixel-topixel analysis. Pixel size is 125 nm. Circles indicate overlapping regions containing statistically significant microdomains (group of pixels) identified by 2D-CWT analysis and nanodomains identified by single pixel analysis. **(C)** Distribution of FRET intensity in control and at time points of forskolin activation of CREB-dependent transcription. The histogram was fitted by two Gaussian distribution. Individual Gaussian distribution components are shown: first distribution (on the left side of each histogram) represents FRET values due to non-specific interaction of overexpressed ECFP and EYFP labeled FRET partner proteins. Second distribution (on the right side of each histogram) represents a specific cAMP (forskolin) responsive FRET between the labeled interacting domains of CREB and CREB binding protein. The ratio of specific FRET peak (second distribution) to

non-specific peak (first distribution) increased from 1.1 in control to 2.5 at 3 and 5 min of forskolin action. Median of specific FRET intensity distribution increased from 900 a.u. in control to 1150 a.u. after 5min of forskolin action. **(D)** 3D reconstruction of cAMP activated microdomains of CREB and CBP interaction in the nucleus of a representative COS1 cells, obtained with 3D confocal microscopy. *Left*: FRET signal before application of cAMP. *Middle*: FRET signal after 10 min of cAMP application (0.25 mM) *Right*: 3D reconstruction of FRET signal calculated as the difference between control and cAMP-induced FRET. Microdomains of CREB and CBP interaction are clearly seen in the nuclei, except nucleoli region.