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Review

Generation and functions of second messengers microdomains

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ABSTRACT

A compelling example of the mechanisms by which the cells can organize and decipher complex and different functional activities is the convergence of a multitude of stimuli into signalling cascades, involving only few intracellular second messengers. The possibility of restricting these signalling events in distinct microdomains allows a fine and selective tuning of very different tasks. In this review, we will discuss the mechanisms that control the formation and the spatial distribution of Ca^{2+} and cAMP microdomains, providing some examples of their functional consequences.

1. Introduction

In its broad meaning, a microdomain can be defined as a localized subcellular region either physically or functionally distinguishable from the surrounding milieu. Microdomains are not rigidly delimited from a spatial point of view, *i.e.*, their confinement is not necessarily obtained through physical barriers such as membranes. Frequently, the term refers to the concept of heterogeneity in the molecular composition of specific regions or, most often, indicates appreciable differences in the concentration of specific molecules within a given environment. These differences are usually dynamic and can be maintained for relatively long periods of time or, on the contrary, be transient and rapidly vanish within milliseconds of their appearance.

Examples of microdomains range from lipid rafts (plasma membrane regions, 5–300 nm in size with a specific lipid (and protein) composition, different from that of the bulk plasma membrane (PM) [1,2]) to signalling events localized in a specific part of the cell. In this review, we will first focus on the mechanisms that govern the formation and modulate the shape and the amplitude of Ca^{2+} microdomains. We will also discuss other forms of microheterogeneity of water-soluble second messenger levels, cAMP in particular, focusing on examples of localized generation and action of this second messengers within membrane-enclosed compartments. Examples of the functional consequences of microdomains in regulating specific cellular processes are also discussed.

2. Ca^{2+} nano/microdomains near the mouth of Ca^{2+} channels

Ca^{2+} is a key intracellular second messenger and it regulates a multitude of cell physiological processes, ranging from exocytosis and contraction (occurring in the μs and the ms range, respectively) to the control of transcription and proliferation (min and h range, respectively) [3–5]. The universality and the versatility of Ca^{2+} as a messenger depends not only on the fact that most Ca^{2+} signals are often transmitted as temporally separate transient oscillations, but also on the fact that they can be organized into spatially distinct domains, allowing a localized regulation of different processes.

The generation of a Ca^{2+} microdomain usually depends on elementary events, such as the opening of one or few Ca^{2+} channels located in either the PM or in the intracellular Ca^{2+} stores [6,7], the endoplasmic/sarcoplasmic reticulum (ER/SR) or the Golgi apparatus. Indeed, upon channel opening, Ca^{2+} promptly flows from the compartment with a higher concentration of the cation (*i.e.*, the extracellular milieu or the lumen of an intracellular store) into that with the lower $[Ca^{2+}]$, the cytosol. Both the extracellular milieu and the intracellular stores function as Ca^{2+} reservoirs, with the former being virtually infinite while the latter is depletable. In a simplified model, once in the cytosol, Ca^{2+} spreads from the mouth of the Ca^{2+} channel towards the more distant areas, generating a gradient that follows Fick's diffusion laws, so that the flux of Ca^{2+} is, for each point, inversely proportional to its distance from the

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source. However, this model is not directly applicable in the cellular context, since it is tremendously complicated by the presence of a highly viscous medium, many immobile and mobile Ca^{2+} buffers, pumps/exchangers that can rapidly remove Ca^{2+}, and different structures/organelles endowed with the ability to take up or further release Ca^{2+}, all players that shape the diffusion gradients. Moreover, the possibility to finely tune the frequency and the temporal extent of the channel opening offers the cells an additional tool to achieve temporally and spatially localized Ca^{2+} microdomains in the proximity of the channels.

As to the Ca^{2+} buffers, they are mainly represented by proteins with the ability to bind Ca^{2+}, by negatively charged phospholipids and some small soluble molecules (as, for instance, ATP) that also contribute to buffering (see also below). In the lumen of the ER/SR, proteins such as calreticulin (CRT) and calsequestrin (CSQ) represent the best studied and abundant Ca^{2+} buffers; they are characterized by a high number of Ca^{2+} binding sites (up to 50 per molecule) and by a relatively low affinity for the cation, that allows the storage and the diffusion of large amounts of Ca^{2+} inside these organelles and its prompt release upon opening of the releasing channels [8–10]. Conditions that alter their expression levels affect the storage capacity and the amount of Ca^{2+} released after stimulation [11,12]. Other proteins, such as GRP78, GRP94 and calnexin (CNX), classical protein chaperones, also contribute to Ca^{2+} buffering in the lumen of the ER [9,13,14]. Much less is known about Ca^{2+} buffering within other organelles, *i.e.*, Golgi [15], secretory granules and mitochondria. As to the latter organelles, an important component of the matrix Ca^{2+} buffering is represented by phosphate that can form different types of complexes with Ca^{2+}, favoured by the alkaline pH of the environment [16].

In the cytosol, the best known Ca^{2+} buffering proteins are parvalbumins, calbindins and calretinins and their expression is highly variable in different cells [17,18]. Ca^{2+} sensors, *e.g.*, molecules endowed with specific Ca^{2+} binding sites such as calcineurin (CN), are expressed at much lower concentrations, but the best known of them, calmodulin (CaM), is ubiquitously expressed and highly concentrated (several μM) in all cells [19]. Last, but not least, since the negatively charged phospholipids are abundant in every membrane, they contribute to the Ca^{2+} buffering capacity, although their affinity for Ca^{2+} is not high. However, the quantitative contribution of these molecules has not been accurately evaluated. It has been calculated that, upon opening of a Ca^{2+} channel, the immobile buffers (such as those associated with the membrane surrounding the channel itself) do not influence the concentration profile of the Ca^{2+} gradient reached at steady state [20]. Indeed, while during the formation of the microdomain they surely protract the time necessary to reach the steady state, once this is obtained they tend to be saturated given that, being anchored to membranes, they cannot by replenished in the ms range. On the contrary, mobile buffers undergo negligible saturation (since they are free to diffuse and to be replaced by the Ca^{2+} unbound species) and thus actively shape the microdomain characteristics [21].

Microdomains are formed with a temporal range of a few tens to hundreds of milliseconds and extend spatially to a few tens-hundreds of nanometres. For a long time, it was thus very challenging to perform Ca^{2+} imaging experiments with sufficient temporal and spatial resolutions at the same time. However, the problem was addressed also by generating mathematical models of the steady-state shape of a Ca^{2+} microdomain. In elegant calculations [21] a number of concepts have been formulated. First, the "strength of the source" (*i.e.*, the flux of Ca^{2+} from the channel given by its current) is a determinant aspect: the larger the flux from the channel, the higher the increase of $[Ca^{2+}]$ above resting values in the microdomain. Second, each buffer has a specific distance from the source where it is maximally effective in its contribution to Ca^{2+} binding and thus in modulating the shape of the microdomains. This distance depends on three different characteristics of the buffer. First, the mean reaction times of the buffer with Ca^{2+} (*i.e.*, its kinetic constants): the faster it binds Ca^{2+}, the higher its chance to capture Ca^{2+} very close to the channel where, according to Fick's laws of diffusion, the speed of the ions flowing towards areas of lower concentration is higher. This implies that fast chelators such as BAPTA or ATP (K_{on} 4.0×10^8 and $5.0 \times 10^8\ M^{-1}\ s^{-1}$, respectively [21]) are particularly effective at changing the flux of Ca^{2+} near the channel, while slow chelators such as EGTA (K_{on} $2.5 \times 10^6\ M^{-1}\ s^{-1}$) are less efficient near the channel and become important only in more distant areas. Second, the buffering power of the specific buffer species (binding ratio), which is the capacity to bind Ca^{2+} at a given $[Ca^{2+}]$: the higher this value (that depends, among other factors, also on the buffer concentration), the higher its ability to dampen the microdomain. Buffers such as BAPTA and EGTA have high buffering capacity, while others (ATP, for example) exhibit a low capacity. Third, the mobility of the buffer (*i.e.*, the diffusion constant D): if a buffer is very mobile, it can promptly carry Ca^{2+} away from the microdomain and be replaced by Ca^{2+}-free buffer, thus maximally affecting the shape of the microdomain.

In light of these considerations, the authors derived a series of deductions. Upon opening of a channel, a Ca^{2+} microdomain is promptly formed and within few hundreds of nm reach a steady-state in <1 ms. Similarly, when the channel closes, the microdomain vanishes almost immediately. In a theoretical scenario, in which the buffer is 1 mM BAPTA, this microdomain can extend for ~150 nm from the mouth of the channel. When the buffering capacity is increased (using a mix of 2 mM ATP, 0.5 mM of an endogenous buffer [22] and 2 mM BAPTA), the distance from the channel up to which an appreciable $[Ca^{2+}]$ gradient is retrieved was calculated to be around 50–70 nm [21].

Whenever studying localized subcellular Ca^{2+} dynamics with the most commonly used chemical Ca^{2+} indicators, the fact that the buffering power and the mobility of the buffer species can modulate the spatio-temporal shape of a microdomain is an aspect that needs to be carefully evaluated. Indeed, many of the probes usually employed for this purpose (for instance, Fura-2 and most of the fluorescent probes of this family) have a significant Ca^{2+} buffering capacity and, being small molecules, are endowed with a relatively high mobility, that can speed up the diffusion of Ca^{2+}; the diffusion constant of the Ca^{2+}-bound (and Ca^{2+}-free) indicators is in fact higher than that of the majority of the endogenous Ca^{2+}-buffers (predominantly represented by proteins, as described above). In other words, the indicators themselves alter the dynamics of the parameter under investigation. Accordingly, the higher the concentration of the Ca^{2+} indicator used, the lower the amplitude of the Ca^{2+} rises; on the contrary, the speed of Ca^{2+} diffusion from the channel mouth and within the cytosol is faster, the higher the concentration of the probe. From this point of view, genetically encoded Ca^{2+} indicators (GECI) [23–25] appear superior, as their concentration hardly exceed a few micromolar (thus their buffering power is generally lower than that of chemical indicators) and their diffusion constant is similar to that of endogenous, proteinaceous buffers. An alternative approach is to use dextran bound Ca^{2+} indicators, that however need to be microinjected in the live cells.

The above considerations are based on mathematical models valid for an idealized cellular situation. In this scenario, the amplitude and the diffusion of the Ca^{2+} microdomains from the channel mouth depend on a number of parameters: the intensity of the Ca^{2+} current, the concentration, affinity, binding kinetics and the chemical nature of the buffers. It is thus predicted that, given the large variability in these parameters among different cell types (and experimental conditions), the Ca^{2+} peaks reached in the microdomains and their diffusion into the bulk cytosol will be very different not only among different cells, but also in different regions of the same cell (*e.g.*, plasma membrane *vs.* intracellular