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Review

Frequency decoding of calcium oscillations ☆

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ABSTRACT

Background: Calcium (Ca^{2+}) oscillations are ubiquitous signals present in all cells that provide efficient means to transmit intracellular biological information. Either spontaneously or upon receptor ligand binding, the otherwise stable cytosolic Ca^{2+} concentration starts to oscillate. The resulting specific oscillatory pattern is interpreted by intracellular downstream effectors that subsequently activate different cellular processes. This signal transduction can occur through frequency modulation (FM) or amplitude modulation (AM), much similar to a radio signal. The decoding of the oscillatory signal is typically performed by enzymes with multiple Ca^{2+} binding residues that diversely can regulate its total phosphorylation, thereby activating cellular program. To date, NFAT, NF-κB, CaMKII, MAPK and calpain have been reported to have frequency decoding properties.
Scope of review: The basic principles and recent discoveries reporting frequency decoding of FM Ca^{2+} oscillations are reviewed here.
Major conclusions: A limited number of cellular frequency decoding molecules of Ca^{2+} oscillations have yet been reported. Interestingly, their responsiveness to Ca^{2+} oscillatory frequencies shows little overlap, suggesting their specific roles in cells.
General significance: Frequency modulation of Ca^{2+} oscillations provides an efficient means to differentiate biological responses in the cell, both in health and in disease. Thus, it is crucial to identify and characterize all cellular frequency decoding molecules to understand how cells control important cell programs.

1. Introduction

The key question in the field of calcium (Ca^{2+}) signaling is by what means this simple ion can regulate such a wide spectrum of cellular processes, including fertilization, proliferation, differentiation, muscle contraction, learning and cell death [1,2]. The answer to this question most certainly lies in the huge spatial and temporal diversity of the signal, since a Ca^{2+} response can exhibit infinite patterns [3]. Through an intricate concert of action between several Ca^{2+} transporters in the cell the cytosolic Ca^{2+} concentration can start to oscillate, much like a radio signal. Specific information can thereby be efficiently encoded in the signal and transmitted through the cell without harming the cell itself [4,5]. Already at the beginning of life, when the sperm injects phospholipase C-ζ into the egg, a slow Ca^{2+} oscillatory wave is traveling the egg that triggers fertilization [6]. Transmitting information using oscillating waves can occur by means of amplitude (AM) or frequency modulation (FM) (Fig. 1). However, Ca^{2+} oscillations *in vivo* are never totally homogenous, but rather have intrinsic variations in oscillation parameters such as frequency and amplitude [7]. This article will review and describe the recent literature on frequency decoding of FM Ca^{2+} oscillations. Thus, spatial aspects of Ca^{2+} signaling, including waves, intercellular communication and microdomains, will not be reviewed here. It should be noted though, that this dichotomy is a simplification since, for example, Ca^{2+} oscillations in mast cells convey transcriptional information only in the neighborhood of CRAC (Ca^{2+} release activated Ca^{2+}) channels [8]. Most studies mentioned in this review employ Ca^{2+} clamping to artificially induce frequency-controlled Ca^{2+} oscillations, either with or without an agonist.

2. Information encoding

Cells are constantly exposed to extracellular stimuli, *e.g.*, mitogens, cytokines, hormones and neurotransmitters, that translate into changes in the cytosolic Ca^{2+} concentration [9]. In addition, environmental cues causing temperature and mechanical stress can result in activation of Ca^{2+} signals [10]. During development, the otherwise stable cytosolic Ca^{2+} level starts to oscillate spontaneously due to largely unknown reasons [11,12]. Depending on the stimuli, that differentially affect Ca^{2+} channels and pumps as well as Ca^{2+} binding proteins, a unique Ca^{2+} signal is induced. The process of building up a unique signal that can be associated with a specific stimulus is called information encoding. In certain circumstances, the stimulus results in persistent oscillatory changes in the cytosolic Ca^{2+} concentration. The biochemistry of such

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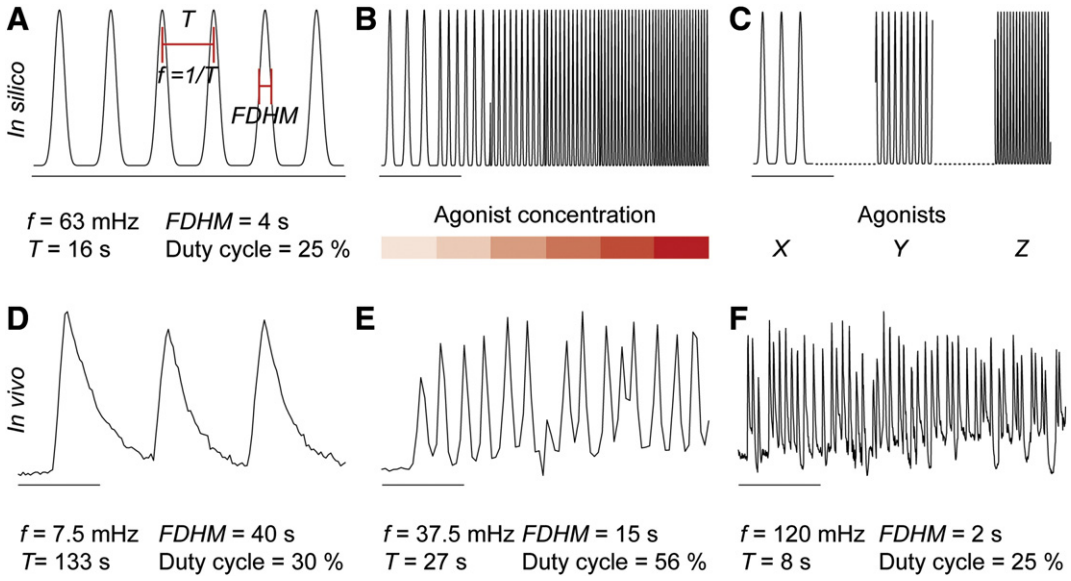

Fig. 1. Frequency modulated Ca^{2+} oscillations. (A) A computer generated (*in silico*) oscillating wave with the parameters: period (*T*), frequency (*f*), full duration half maximum (FDHM), and duty cycle is depicted. (B) Oscillating wave frequency modulated by agonist concentration. (C) Oscillating wave frequency modulated by the different agonists *X*, *Y*, and *Z*. Three single cell Ca^{2+} recordings of a Fluo-4/AM-loaded neuroblastoma cell (D), HeLa cell (E), and cardiac cell (F) with the parameters *T*, *f*, FDHM, and duty cycle stated. Scale bars are 100 s.

Ca^{2+} oscillations has been reviewed elsewhere [13]. The oscillatory frequency can vary from tens of Hz in neurons to tens of mHz in non-excitable cells [14]. Occasionally the frequency is proportional to the amount of stimulus to which the cell is exposed [15–19].

3. Information decoding

Decoding is used by the cell to interpret the information carried by the Ca^{2+} oscillation [20]. This information deciphering occurs when one or several intracellular molecules sense the signal and change their activities accordingly. The process is similar to electromagnetic radiation being received by an antenna on a radio and translated into sound. Mathematical modeling of a generic Ca^{2+} sensitive protein has shown that it is possible to decode Ca^{2+} oscillations on the basis of the frequency itself, the duration of the single transients or the amplitude [21]. Detailed models of real protein-decoders have also been reported [22–24].

At the molecular level, the Ca^{2+} oscillatory frequency regulates the activity of the decoder. The molecular mechanism of decoding is thought to include the on-and-off kinetics of Ca^{2+} binding to kinases and phosphatases, which respectively activate and inactivate target proteins. If the oscillation frequency is much lower than the typical on-off-frequency, no integration will occur and the signal is simply decoded as a sum of single transients. Consequently, maximum frequency sensitivity should be observed for signals with duty cycles between 0 and 0.5. The duty cycle is defined as the ratio of the spike duration to the period (Fig. 1). Oscillating signals are more effective in activating target effectors than constant signals when Ca^{2+} is bound cooperatively and with low affinity (dissociation constants around the peak Ca^{2+} concentration). However, with duty cycle values below 0.5, this requirement is less strict [21]. Several proteins appear to have such characteristics, including phospholipase Cδ [25,26], protein kinase Cβ [27], calmodulin [28], Ca^{2+}- and calmodulin-dependent protein kinase II (CaMKII) [29], calcineurin [30], troponin C [31] and the mitochondrial Ca^{2+} uniporter [32]. Salazar et al. define pure frequency encoding as changes in frequency with constant duty cycle, whereas biological frequency encoding is when the duty cycle varies with the frequency as the spike duration remains constant [21]. Both types of frequency encoding as well as amplitude encoding are more efficient than constant signals. In almost all studies mentioned here (except from [33]), cells exhibit Ca^{2+} oscillations of variable frequency but constant duration, thus modeling biological frequency encoding.

4. NFAT

The transcription factor NFAT (nuclear factor of activated T-cells) has been shown to function as a decoder of Ca^{2+} oscillations [34] and to exhibit working memory properties [35]. In its inactivated state, it is phosphorylated on multiple sites and kept stable in the cytosol [36]. Upon activation by the phosphatase calcineurin, which is modulated by Ca^{2+} binding and calmodulin, NFAT is dephosphorylated and translocated to the nucleus to become transcriptionally active. Upon re-phosphorylation in the nucleus, NFAT can be transported back to the cytosol. The kinetics of the nuclear export varies among different NFAT subtypes [37]. Dolmetsch et al. were first to use frequency-controlled artificially induced Ca^{2+} oscillations on Jurkat cells to show that the NFAT activity is positively correlated with frequencies in the range of 2.5–10 mHz, with a duration of 50 s (duty cycle 0.125–0.5) [34]. The same study showed similar frequency decoding properties for Oct/OAP and NFAT [34]. Interestingly, the NFAT transcriptional activity is both negatively and positively correlated with different Ca^{2+} frequencies in RBL-2H3 cells [38]. The maximum NFAT activity is present at the Ca^{2+} frequency of 16.7 mHz (duty cycle 0.33) with decreasing activity down to 2 mHz and up to 33 mHz, with a duration of 20 s (duty cycle 0.04–0.66). In BHK and Jurkat cells, the frequency of Ca^{2+} oscillations was positively correlated with dephosphorylation and translocation of NFAT4 in the Ca^{2+} frequency range of 1–11 mHz, with a duration of 30 s (duty cycle 0.03–0.3) [35]. The maximum efficiency was gained at 2.8 mHz (duty cycle 0.08) and an oscillatory Ca^{2+} signaling above 5.6 mHz results in stronger nuclear translocation than a sustained Ca^{2+} increase. Higher frequencies of Ca^{2+} oscillations in a genetic model of Noonan syndrome with cardiac myocytes show less NFAT transcriptional activity in the frequency range of 30–190 mHz, with a duration of 4–8 s (duty cycle 0.24–0.76) [39]. However, here no measurements were performed between 30 mHz and 140 mHz, where a maximum might be. By contrast, another study on rat neonatal cardiomyocytes shows that the NFAT activity is positively correlated with Ca^{2+} frequencies in the range of 17–83 mHz (duration 5 s) [40]. In an *in vitro* model of cardiac cell agonist induced hypertrophy, the cell area positively correlated with frequencies in the range of