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Bifurcations and limit cycles in cytosolic yeast calcium☆

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ABSTRACT

Calcium homeostasis is a fundamental cellular process in yeast. The regulation of the cytosolic calcium concentration is required for volume preservation and to regulate many vital calcium dependent processes such as mating and response to stress. The homeostatic mechanism is often studied by applying calcium pulses: sharply changing the calcium concentration in the yeast environment and observing the cellular response. To address these experimental investigations, several mathematical models have been proposed to describe this response. In this article we demonstrate that a previously studied model for this response predicts the presence of limit point instabilities and limit cycles in the dynamics of the calcium homeostasis system. We discuss the ways in which such dynamic characteristics can be observed with luminometric techniques. We contrast these predictions with experimentally observed responses and find that the experiments reveal a number of features that are consistent with modeling predictions. In particular, we find that equilibrium cytosolic concentrations have a sharp change in behavior as pulse size changes in the micromolar range. We show that such change is consistent with the presence of limit point instabilities. Additionally, we find that the response of synchronized yeast cells to millimolar range pulses is non-monotonic in its late stages. This response has characteristics similar to those associated with limit cycles.

1. Introduction

The budding yeast, *Saccharomyces cerevisiae*, is one of the best studied biological systems thanks to its relatively small genome and a readily manipulable life-cycle. In addition to being of intrinsic interest, the study of yeast is important as part of medical research of human disease [1,2] as the yeast genome includes major functionally of larger organisms [3]. In particular, yeast has become an important model system for studies of calcium mediated processes in mammalian myocardiac cells [4].

The budding yeast *Saccharomyces cerevisiae* utilizes calcium as a general second messenger, generating calcium pulses and fluxes in response to a variety of signals such as hyperosmolarity, hypo-osmolarity, cell stress, glucose, and alpha factor (reviewed in [5–7]). Calcium is transported from the extracellular media into the cell, where it is stored in membrane bound organelles. First identified were two plasma membrane calcium channel systems, HACS (high affinity calcium influx system) identified as Cch1p/Mid1p [8,9] and the as yet uncloned LACS (low affinity calcium influx system) [10] and these were further characterized as to their physiological activities and specificities. Additional calcium channels on the yeast plasma membrane have been proposed. These include channel X [11], the glucose induced channel [12], a calcium stimulated magnesium channel [15], and a channel induced under a stress-inducing amiodorone treatment [13,14]. Once high levels of calcium are transported inside the yeast cell, it activates a signal transduction cascade, including calmodulin, calcineurin, and a transcription factor, Crz1p, and then is quickly sequestered away in the vacuole and the Golgi apparatus, to be stored and used for subsequent signaling [6]. A number of works have sought to mathematically describe this calcium homeostatis network [4,11,15,16] with the aim of finding further insight into its structure and features. Other related modeling works have addressed different calcium mediated process in yeast and in similar systems [17–21].

This article has three different but integrated goals. First, to present an analysis of a previously published model of yeast calcium homeostasis that examines the presence of limit cycles and bifurcation features in it. Second, to discuss how these features could be identified in experimental measurements that do not directly observe calcium concentration in organelles of individual cells, but rather quantify the cytosolic calcium of a yeast population. Such measurements are relevant

☆ Yeast calcium bifurcations.

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as they are common in practice and easy to carry out. Finally, to discuss concrete experimental results in light of the possible presence of bifurcations and limit cycles predicted by the model.

The results of this investigation show that bifurcative or limit cycle behavior might be in fact possible. While no direct evidence is available, our experimental observations point to regions in the space of controllable parameters where these features might be found. The nonlinearity of the cytosolic calcium response to external calcium pulses, and the long term non-monoticity of the cytosolic calcium after such pulses do suggest the existence of a complex set of states in the yeast calcium-dependent systems.

We organize the paper as follows: in Section 2 we present an overview of the model of Cui and Kaandorp [11]. As the model encompasses many features of calcium-related cell activity, a full description of all the details of the model would be too lengthy. Instead, we summarize its key features, state the notation used, and present the resulting equations. In Section 3 we present an analysis of the features of the model. In particular, we exhibit the presence of bifurcations and limit cycles for a set of parameters of the model, that suggest that these features might be observable in experimental systems. Section 4 discusses the difficulties of directly observing these features and provides a guide to indirect indications of their presence. Section 5 describes the experimental methods used to investigate the possible presence of these features and Section 6 present the experimental results. We close with a discussion of these results in Section 7.

2. Mathematical model

In this section we consider the model developed by Cui and Kaandorp [11] to explore the dynamics of calcium homeostasis. The original publication focused on the features of the transient response of the cytosolic calcium to a sharp increase in external calcium. The model considers the population and activity of a number of proteins known to play key roles in calcium homeostasis. In previous work by our group [16], the model was extended to consider the responses of mutants lacking some of these key proteins. In this article we continue the investigation of the model, focusing on the asymptotic states that it predicts.

The main goal of the model [11] is to describe the response of the internal free calcium concentration $[Ca]_{cyt}$, which we now simply call x, upon a steep change in the external calcium concentration $[Ca]_{ex}$, referred to from now on as C_{ex}. The model addresses both the flow of calcium into different compartments of the cell and its interactions with specific molecules as well as the downstream effect of these interactions in gene expression. Our detailed description of the model is broken into a number of subsections. We first present, in Section 2.1, an overview of the model and follow with the resulting calcium flux balance equation in Section 2.2. We next describe, in Sections 2.3–2.5, the interaction of calcium with key molecules that lead to downstream effects. The next Section 2.6, presents the components of the model associated with the production of key proteins that in turn control the calcium flux. Section 2.7 discusses the model for channel X, through which calcium ions enter the cytosol. The resulting set of equations is summarized in Section 2.8. The final Section 2.9 briefly discusses a number of possible criticisms of the model.

The construction of the model and validation of its many components required extensive discussion in the original publication. In this article we do not wish to replicate the detailed arguments leading to it, but focus only on a description of its key features. We urge the interested reader to review the publications that have previously discussed the model [4,11,16].

2.1. Calcium homeostatis

The central features of the model are summarized in the control (Fig. 1) and flux (Fig. 2) diagrams. The setting of the model is an

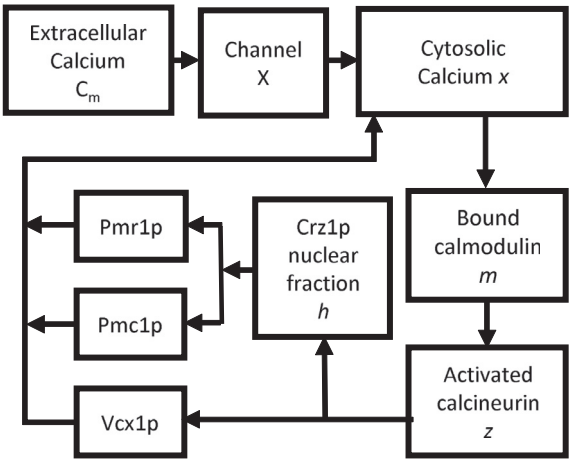

Fig. 1. The control diagram indicates the feedback mechanisms considered by the model.

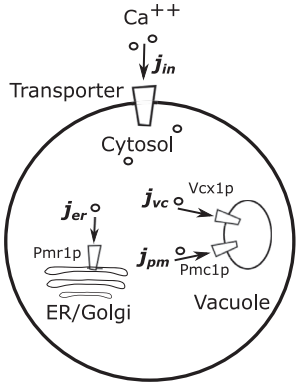

Fig. 2. The diagram shows the calcium fluxes considered by the model between different cell compartments.

experiment in which yeast cells are exposed to an environment with a time-dependent extracellular calcium concentration C_{ex}. The simplest form of the experiment assumes that the external calcium changes sharply from an initial low concentration to a new elevated value; that is, the yeast is presented with a calcium pulse. After the pulse, cytosolic calcium is acquired from the extracellular environment through a transporter, called here channel X, whose protein components are as yet unknown [11]. The elevated cytosolic calcium concentration can be reduced by sequestration into the vacuole through Vcx1p [22], a calcium/proton exchanger, and the calcium ATPase Pmc1p and into the endoplasmic reticulum and Golgi apparatus through the calcium/magnesium P type ATPase Pmr1p [6].

In this model the flux rates of calcium into the organelles are subject to feedback. It is assumed, in particular, that the abundances of channels Pmc1p and Pmr1p are affected by calcium concentration. Cytosolic calcium concentration is sensed by calmodulin which, when associated with three calcium ions, can bound to calcineurin to activate it. Activated calcineurin, in turn, controls the level of activity of the transcription factor Cr1zp [5]. Finally, this transcription factor controls the synthesis of Pmc1p and Pmr1p [23,24]. The model also considers a negative feedback relation between calcium concentration and the population of the Vcx1p channel [22,25], though its precise molecular mechanism is not specified in the level of detail as the feedback system for Pmc1p and Pmr1p.

2.2. Calcium fluxes

Given the assumptions of the model, the cytosolic calcium concentration changes can be traced back to the injection of external ions