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Mathematical modeling of metal ion homeostasis and signaling systems

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Chapter 7 Final Discussion and Future Work

The modeling work presented in this thesis is done for metal ion homeostasis and signaling systems in various organisms, including both prokaryotes (e.g., *E. coli*) and eukaryotes (yeast, mice). More specifically, Chapter 2 and 3 are about yeast calcium homeostasis and signaling, Chapter 4 is about complex calcium signaling network in cardiac myocytes in mice, Chapter 5 is related to zinc homeostasis system in *E. coli* and Chapter 6 is about the general design principles of these metal ion homeostasis and signaling networks.

The work demonstrated in Chapter 2 and 3 shows that yeast has both fast feedback pathway (i.e., the quick inhibition on the influx transporters possibly by calmodulin, see the dashed lines in Fig. 3.1B) responsible for the short-term calcium response and slow feedback pathway (i.e., the calcineurin-dependent feedback pathway, see Fig. 2.1C) to regulate the long term calcium homeostasis. This finding in yeast cell is consistent with the specific responsiveness of calcineurin to sustained, low frequency calcium signals as found in animal cells ([224], also see Section 4.2.3). In the short-term response, *Vcx1* is the main transporter responsible for rapidly sequestering cytosolic calcium (please compare Fig. 3.2B with Fig. 3.2C) whereas in the long-term calcium homeostasis, *Pmc1* becomes the most critical transporter because calcineurin is activated to increase the expression of *PMC1* and *PMR1* and inhibit the activity of *Vcx1*.

As mentioned in Section 1.3.4.6, Scrase-Field *et al.* (2003) ever proposed that calcium may work merely as an essential chemical switch rather than an encoder of signal specificity as the calcium signature hypothesis assumes [189]. Their arguments for the calcium switch proposal include experimentally found phenomena in plant calcium signaling such as similar calcium signatures with different end responses, similar end responses with different calcium signatures. For example, osmotic shock and salt shock induce very similar calcium signatures within both the cell and the whole plant. If calcium signature hypothesis is right, these two stimuli should induce the same end responses. However, the calcium signature induced by non-ionic osmotic shock does not induce the SOS3-SOS2-SOS1²⁷ complex as the signature induced by salt stress does [189].

Similar phenomena are also experimentally found in yeast cells. For example, Wiesenberger *et al.* (2007) reported that Mg^{2+} starvation and Ca^{2+} stress can induce very similar cytosolic calcium transients in yeast (this is also confirmed by our simulations, see Section 3.3.2.4) and a striking portion of genes including *ENAI* (encoding a P-type ATPase sodium pump) and *PHO89* (encoding a sodium/phosphate cotransporter) up-regulated under Mg^{2+} depletion are also induced by Ca^{2+} stress. However, lots of other genes (e.g., *SUL1*, *ARA1*, *MDH2*, *STF2*) up-regulated under Mg^{2+} depletion are not induced by Ca^{2+} stress [234].

²⁷ In response to the salt shock, both SOS3 and SOS2 are important in stimulating the plasma membrane Na^+/H^+ exchange activity of SOS1. SOS3: Salt Overly Sensitivity3.

As stated in Section 1.3.4.6 and also above, both calcium signature hypothesis and chemical switch seem to have their respective supporting experimental evidence. Thus it will be quite important to discriminate the cases in which calcium is responsible for signal specificity with those in which it only functions as a switch. From the viewpoint that the end response is usually the result of the cross-interaction of multiple signaling pathways, we can understand more easily why the signal specificity can be encoded by signaling components other than calcium. Actually our work presented in Chapter 4 shows an example of how different stimuli can cause their respective end response through the complex cross-interaction of different signaling pathways (e.g., PO induces cardiac hypertrophy by activating both the calcium-calcineurin-NFAT pathway and the BMK1/ERK5 signaling pathway [95] whereas the BMK1/ERK5 signaling pathway is not activated in the case of CaN* overexpression). The eventual accurate mathematical approximation of the calcium signaling systems will help us uncover the mystery of encoding signal specificity.

On one hand, the quantitative agreement of our simulation results with the corresponding experimental data (e.g., compare Fig. 3.4A-B with Fig. 3.2B-C; also see Fig. 5.2, Fig. 5.3d, Fig. 5.9) and the ability of our models of predicting certain mutant behavior (e.g., see Fig. 2.4a-b, Fig. 3.4B, Fig. 4.2b) show the usefulness of mathematical modeling. On the other hand, we need to realize that to accurately approximate the studied systems, lots of work remain to be done. Many missing components of the relevant networks need to be identified, a number of rate parameters need to be determined and the exact *in vivo* concentrations of many relevant proteins need to be quantified, which illustrates the great challenges imposed by systems biology (see Section 1.1.3). Close cooperation between biologists and computational scientists through iterative procedure of systems biology (experiment→model→experiment) is necessary for devising realistic models (see Section 1.2.1) [72]. The work presented in Chapter 3 gives an example showing effective cooperation between biologists and modelers indeed can help detect the existence of new components of the metal ion homeostasis and signaling networks and to push forward our understanding of these complex systems.

Compared with those systems in more complicated organisms such as mice and human, metal ion homeostasis and signaling systems in simpler organisms (e.g., yeast and *E. coli*) are easier to approximate. As shown in Chapter 2 and 3, yeast cell (*Saccharomyces cerevisiae*) has an elaborate calcium homeostasis/signaling system whose components (except the H⁺/Ca²⁺ antiporter) have corresponding homologues in animal cells (see Table 1.3). Surprisingly, most of these factors operate similarly in human cells, for example, NFAT translocation in human cells is strikingly similar as Crz1 translocation in yeast [208], MCIP signaling in human cells is similar as Rcn1 signaling in yeast [100,116]. Thus the understanding of calcium homeostasis/signaling system in the budding yeast *Saccharomyces cerevisiae*, a simple organism that affords powerful genetic and genomic tools, can be a shortcut to help understand calcium homeostasis/signaling systems in human and treat relevant human diseases such as pathological cardiac hypertrophy and heart failure.

In order to gain a more complete understanding of calcium homeostasis/signaling system in *S. cerevisiae* as shown in Fig. 3.1A, the first critical thing is that we need to identify the missing components of the network. Genome-wide high-throughput screens and comparative genomics can be helpful for locating the possible candidates [40,76,81]. Recently, proteomics has developed into such stage that it can determine the cellular response to any perturbation at the level of protein activation (e.g. phosphorylation) [3,45,54,121,160]. Thus mass-spectrometry-based proteomics can be a very powerful technique for searching the missing components and for detecting and determining the protein interactions as well. Moreover, the theory of network motifs may help determine the local network structure. The second critical thing is that we need to quantify the concentrations of proteins and measure the rate constants. Again, the former task can be achieved by mass-spectrometry-based proteomics [45,54]. In order to measure the rate constants, various existent methods (e.g., surface plasmon resonance analysis [171]) can be used. The third critical thing is that we need to accurately approximate the electrophysiological properties of ion channels (e.g., Cch1-Mid1 [48,132]). Electrophysiological recordings of ion channels in the plasma membrane of live yeast cells have been proven to be quite difficult [174]. An alternative way to achieve such task is to express the corresponding genes into mammalian cells where electrophysiological recordings become much easier. Finally, mass-spectrometry-based proteomics can also be used to measure the nucleocytoplasmic transport of relevant proteins (e.g., Crz1 and calcineurin) [161,216]. As mentioned before, the greatest advantages of yeast are that this simple unicellular organism is so small that certain spatial effects (e.g. diffusion) can be neglected and that it affords powerful genetic and genomic tools as well. As shown in Chapter 2 and 3, functional assays based on gene-knockout techniques provide powerful check for the validity of the models [99]. Effective collaborations among scientists who are proficient in genetics, proteomics and computational science via high-throughput experimental and computational methods will not only result in the eventual completion of the whole yeast calcium homeostasis/signaling system and the understanding of its dynamics in the near future, but also help push forward our understanding of the calcium homeostasis/signaling systems in all other organisms.

In order to further our understanding of the zinc homeostasis in *E. coli*, the most critical thing is to identify the intracellular zinc chaperone which is very likely to exist (see the second last paragraph in Section 5.4). Again, mass-spectrometry-based proteomics and genome-wide high-throughput screens can be used to search the possible candidates. Once the zinc chaperone is identified, the next step will be to measure the interactions between the zinc chaperone and the membrane transport proteins (ZnuABC, ZupT, ZntA and ZitB) and the interactions between the zinc chaperone and the metalloregulatory proteins (ZntR and Zur). Since similar work has been done already for the copper homeostasis system in *E. hirae* [202], the same technical equipments and methods used there (e.g., surface plasmon resonance analysis [171]) can be used to measure the kinetics of these interactions. Then we need to further take into consideration the zinc storage and zinc using proteins in the *E. coli* cell and quantify the concentrations of the relevant proteins, DNAs and mRNAs. Finally, the subtle details of relevant regulations (e.g., proteolysis which has been proven to play a role [172]) need to be further investigated. In this way, step by step we will acquire a complete map of the zinc homeostasis system in

E. coli and the full understanding of its dynamics.

As mentioned before, all the models presented in this thesis are represented in nonlinear ODEs which ignore the spatial effects and stochastic effects of biological events (see Section 1.2.2) [69]. In some cases when the stochastic effects are not negligible, stochastic simulation methods are needed. For example, Monte Carlo simulation methods have been used to simulate the stochastic gating of ion channels which can be expressed as an ensemble of Markov processes [69,197]. In some other cases when the spatial effects are important, partial differential equations become more amenable tool for approximating biological systems [65,151]. For example, PDE models are necessary to capture the spatial effects of calcium signaling such as calcium sparks and waves in animal cells [69,71,197]. Moreover, to simulate complex calcium signaling events as those in embryogenesis (see Fig. 1.3), multi-scale modeling based on PDE modeling becomes inevitable (see Section 1.2.3). For example, if we want to build a comprehensive model for simulating the hypertrophic growth of mammalian heart in response to certain hypertrophic stimuli such as pressure overload, we need to extend the intracellular calcium signaling model presented in Chapter 4 to an organ-level model and then couple it with a spatial growth model of heart. Finally, calcium signaling has been proven to happen within nanodomain of calcium sources [129]. In order to capture the subtle details of these nanodomain signaling events, particle-based modeling techniques such as molecular dynamics become inevitable [75,187]. In the past decades, molecular dynamics methods have been used to simulate the gating of ion channels and help gain important insights into the underlying mechanisms [19]. In these cases, more computational power is usually demanded and special techniques (including new mathematics and new computational methodologies) need to be developed to tackle great difficulties such as the highly irregular boundaries.