Mathematical modeling of metal ion homeostasis and signaling systems

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Chapter 6 Network Motifs and Their Functions[25]

6.1 Introduction

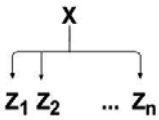
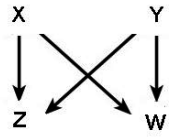
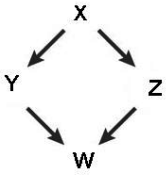
Networks arise naturally in biology for describing the intricate interactions of biological entities. To uncover the design principle of dauntingly complex networks and understand their dynamics, Uri Alon and his colleagues (2002) defined "network motifs" which can be thought of as recurring circuits of interactions and regarded as the building blocks of complex networks [193]. Just as each door or window, which is the building block of normal buildings, has its specific structure and functions, each network motif has its specific structure and is believed to carry out specific information-processing functions. Since these networks motifs are typically small size circuits of interactions and are relatively easy to analyze using mathematical models, the understanding of the specific functions of network motifs has been believed by Alon *et al.* and many other people to be a nice way to help illuminate the dynamics of much more complex systems in which each motif appears [10,11,115].

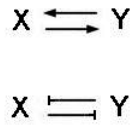
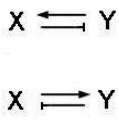
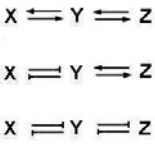
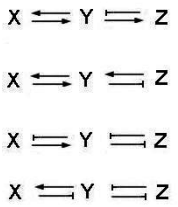
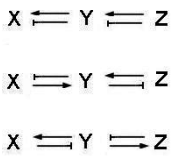
Network motifs were first systematically defined in the transcription network of *E. coli* and have later been found in organisms from bacteria and yeast to plants and animals. An incomplete summary of network motifs and their functions is shown in Table 6.1.

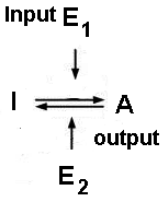
Table 6.1: Network motifs and their functions.

Name		Pattern	Functions	Description
Auto-regulation	Positive autoregulation (PAR)		In transcription networks, PAR slows down the response time of gene circuits and can usually enhance cell-cell variation [10,11].	Component X regulates itself.
	Negative autoregulation (NAR)		In transcription networks, NAR speeds up the response time of gene circuits and can reduce cell–cell	

[25] This Chapter is based on: Jiangjun Cui and Jaap Kaandorp, The modulation on the properties of signaling cycle motif by an inhibitor (in preparation).

			variation in protein levels [10,11].	
Feed-Forward Loop (FFL)	Coherent type-1 FFL (C1-FFL)		In transcription networks, the C1-FFL is a 'sign-sensitive delay' element and a persistence detector [10].	Component X regulates component Y and both of them regulate Z.
	Incoherent type-1 FFL (I1-FFL)		In transcription networks, the I1-FFL is a pulse generator and response accelerator [10].	
	Other FFLS	See Fig. 6.1	Not totally defined	
Single-Input Module (SIM)			In transcription networks, SIM allows coordinated expression of a group of genes with shared function. It can generate a temporal expression program, with a defined order of activation of each of the target promoters [10,11,193].	Component X regulates a group of other components (Z_1, Z_2,…, Z_n).
Bifan			Not well defined [109]	Component X regulates both Z and W. At the same time, component Y regulates both Z and W.
Diamond			Not totally defined	Both components Y and Z are regulated by X and at the same time,

				both Y and Z regulate W.
Feedback Loop with Two nodes	Positive feedback loops (PFL)		In signal transduction networks, PFL amplifies the signal and results in slower response. It can also lead to bistability and hysteresis [115].	Components X and Y regulate each other. In PFL, both X and Y positively regulate each other; or both of them negatively regulate each other. In NFL, only one regulation is positive and the other one is negative.
	Negative feedback loops(NFL)		NFL helps maintaining homeostasis and plays important role in signal adaptation or desensitization to sustained stimulation. It functions as noise filters and accelerates signal responses [115].	
Coupled Feedback Loops	Two positive feedback loops (PP)		PP induces a slower but amplified signal response and enhances bistability [115].	Components X and Y regulate each other. At the same time, Y and Z regulate each other.
	A positive feedback loop and a negative feedback loop (PN)		PN guarantees reliable decision-making by properly modulating signal responses and effectively dealing with noise [114,115].	
	Two negative feedback loops (NN)		NN enhances sustained oscillations and homeostasis [115].	

Signaling Cycle (SC)		SC can amplify the response to a certain stimulus and provide additional sensitivity in biological control. (Typically, the concentration of E_1 is regarded as input and the concentration of active protein is regarded as output) [83,84]	Enzyme 1 (E_1, a kinase) activates a protein (I denotes the its inactive state and A denotes its active state) whereas Enzyme 2 (E_2, a phosphatase) deactivates the protein.

Although numerous papers have discussed network motifs and their functions in various kinds of networks including transcription networks (sensory transcription networks and developmental networks), neuronal networks and signal transduction networks, there is no systematic study reported for discussing networks motifs and their functions in metal ion homeostasis and signaling systems. The aim of this Chapter is to present a preliminary study on this topic. Hopefully such a study will help us to gain some insight into the general designing principles of metal ion homeostasis and signaling systems.

6.2 Network Motifs in Metal Ion Homeostasis and Signaling Systems

As mentioned in Section 1.3.3.2 and 1.3.3.3, metal ion homeostasis systems frequently contain transcriptional regulations on various proteins (ion channels or transporters, metal-using proteins, chaperones and regulatory proteins). Moreover, metal ion signaling systems are specific types of signal transduction systems. Therefore, it is not surprising that many network motifs found in the transcription networks and signal transduction networks also exist widely in the metal ion homeostasis and signaling systems. Here we will give a number of examples and then mainly focus on the signaling cycle motif and study the modulation on its properties by an inhibitor using mathematical model.

6.2.1 Examples of Network Motifs

Autoregulation motif is the simplest motif firstly defined in transcription networks. Positive autoregulation occurs when a transcription factor X activates the transcription of its own gene. In contrast, negative autoregulation occurs when X represses the

transcription of its own gene [10,11,193]. In metal ion homeostasis and signaling systems, many central transcriptional regulators have autoregulations. For instance, the activator Zap1 in yeast zinc homeostasis system (see Fig. 1.2) has positive autoregulation [133]. Fur, the critical repressor in the iron homeostasis system of *E. coli*, has weak negative autoregulation although Fur concentrations do not change significantly in response to iron availability [55]. The repressor CopY in *E. hirae* copper homeostasis system represses the transcription of its own gene [202]. However, no autorgulation of Zur, the critical repressor in the zinc homeostasis system in *E. coli*, was observed [167].

The second important family of network motifs is the feedforward loop. Because each of the three regulatory interactions (see Table 6.1, the regulations of X on Y, Y on Z and X on Z) in the FFL can be either activation or repression, there are totally eight possible structural types of FFL which can be classified into two groups (coherent and incoherent FFLs) as shown in Fig. 6.1 [10,11].

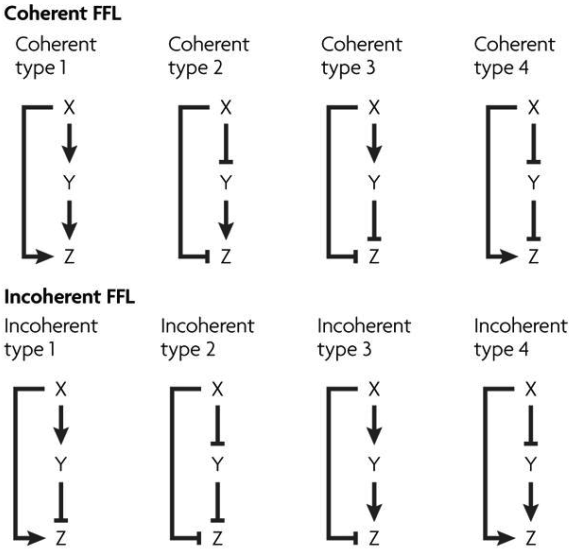


Figure 6.1. Coherent and incoherent FFLs. In coherent FFLs, the indirect regulation of X on Z through Y is coherent with its direct regulation on Z. For example, in coherent type 1 FFL, X activates Y and Y activates Z, thus the indirect regulation of X on Z through Y is activation, which is coherent with the directly activating regulation of X on Z (this figure is taken from [11]).

FFL appears in hundreds of gene systems in various organisms. However, in metal ion homeostasis and signaling systems, FFL is not common. One example of FFL can be found in the zinc homeostasis system in *S. cerevisiae* (see Fig. 1.2), in which cytoplasmic zinc regulates the membrane concentration of Zrt1 both directly (excessive cytoplasmic zinc results in the inactivation of Zrt1 through endocytosis and vacuolar degradation) and indirectly (through Zap1) [133].

The third family of network motifs, SIM motif, exists extreme widely in metal ion and homeostasis and signaling systems because in these systems, it is very common that a single regulator has multiple targets. A good example of SIM motif found in yeast zinc homeostasis system has been described in Fig. 1.7d. Another SIM motif example is that Crz1, the central transcription factor in yeast calcium homeostasis/signaling system, regulates multiple target genes including *PMC1* and *PMR1* [208]. There are many other examples, for instance, in *E. hirae* copper homeostasis system, CopY represses the expression of genes encoding CopA (the uptake ATPase), CopB (the efflux ATPase), CopZ (the chaperone) and itself [202].

Feedback loop motifs are very common in metal ion homeostasis systems. In addition to the example described in Fig. 1.7b, here we give more examples of feedback loop motifs as shown in Fig. 6.2.

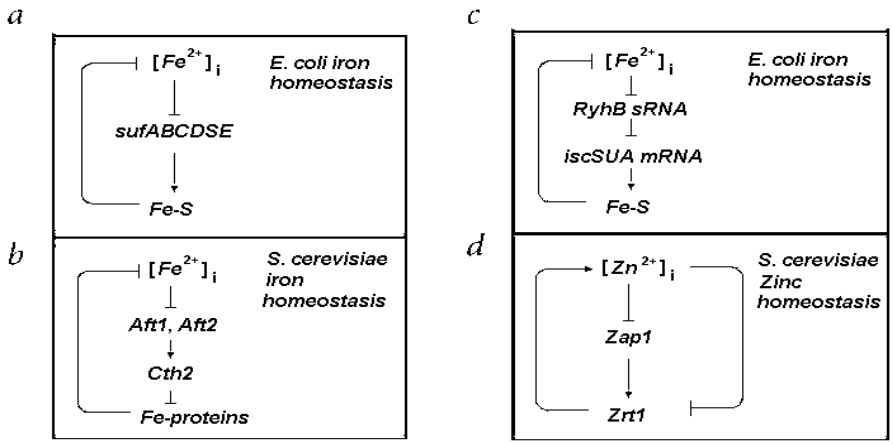


Figure 6.2. More examples of feedback loop motifs. (a). An example of feedback loop motif found in *E. coli* iron homeostasis system. Cytoplasmic Fe^{2+} (i.e., $[Fe^{2+}]_i$) in *E. coli* represses the *suf* operon (encoded by *sufABCDSE*) which is involved in [Fe-S] cluster formation [191]. (b). A feedback loop example found in *E. coli* iron homeostasis system. Cytoplasmic Fe^{2+} in *E. coli* represses a small RNA (sRNA) named as RyhB, which facilitates the degradation of *iscSUA* transcripts. iscSUA are parts of the *isc* operon (encoded by *iscRSUA*) which are involved in [Fe-S] cluster formation [191]. (c). A feedback loop motif example found in the iron homeostasis system in *S. cerevisiae*. In response to iron starvation, the iron-responsive transcription factors Aft1 and Aft2 activate Cth2 which facilitates the degradation of mRNAs encoding Fe-using proteins [142]. (d). A coupled negative feedback loop motif example found in the zinc homeostasis system in *S. cerevisiae*. Under zinc-limiting conditions, zinc-responsive transcription factor Zap1 activates Zrt1. Moreover, excessive cytoplasmic zinc results in the inactivation of Zrt1 through endocytosis and vacuolar degradation [202].

Very frequently, biological cells use coupled feedback loop pathways for the regulations of metal ion and homeostasis and signaling systems. For example, the feedback loops shown in Fig. 1.7b and Fig. 6.2a-c are all coupled with each other and function together to maintain iron homeostasis in *E. coli*. Fig. 6.2d shows a good example of coupled negative feedback loops motif (i.e., coupled NN motif; for the definition of NN, please

see Table 6.1) found in the yeast zinc homeostasis system. More examples of PN and NN network motifs are shown in Fig. 6.3.

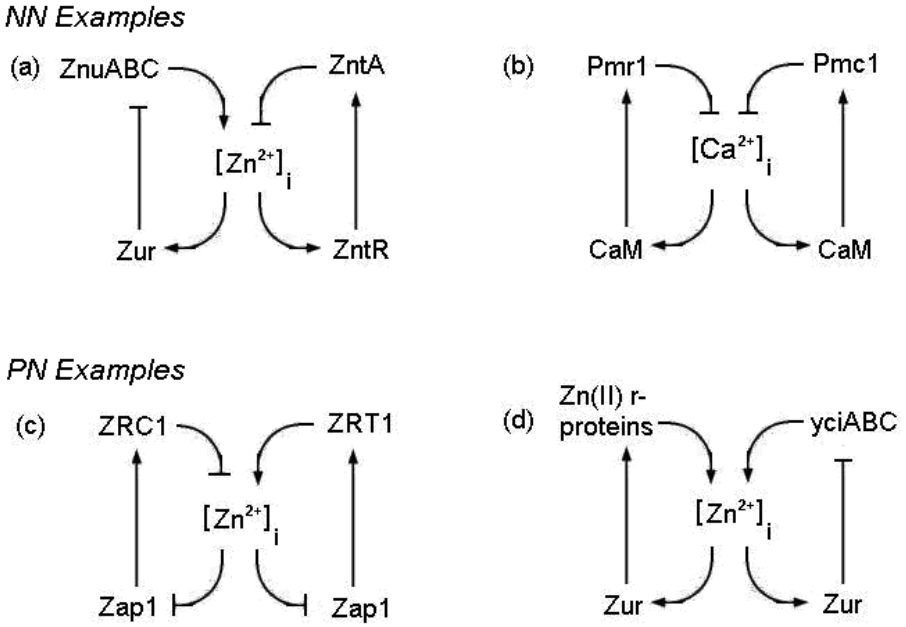


Figure 6.3. Examples of coupled feedback loops (NN and PN) motifs. (a). NN example found in *E. coli* zinc homeostasis system: the Zur-ZnuABC pathway is a negative feedback loop (NFL) and the ZntR-ZntA pathway is another NFL [163,172]. (b). NN example found in *S. cerevisiae* calcium homeostasis system: the CaM-Pmc1 pathway (more precisely, CaM-calcineurin-Crz1-Pmc1 pathway) is one NFL and the CaM-Pmr1 (more precisely, CaM-calcineurin-Crz1-Pmr1 pathway) pathway is another NFL [51,132]. (c). PN example found in *S. cerevisiae* zinc homeostasis system: the Zap1-ZRC1 pathway is a positive feedback loop (PFL) and the Zap1-ZRT1 pathway is a NFL [202]. (d) PN example found in *Bacillus subtilis* zinc homeostasis system: the Zur- Zn(II) r-proteins pathway is a PFL because Zur promotes mobilization of Zn(II) stored in Zn(II) r-proteins. In contrast, the Zur-yciABC pathway is a NFL [149]. CaM: calmodulin; yciABC: Zinc uptake transporter; Zn(II) r-proteins: Zn(II) containing ribosomal proteins.

6.2.2 Signaling Cycle Motif

Signaling cycle motif (see Table 6.1) is a cycle of covalent modification which is a ubiquitous building block of signaling pathways [83,84,213]. For example, in the Ca^{2+}-calcineurin-MCIP-NFAT signaling network shown in Fig. 4.1, there are 8 enzyme catalytic binding reactions (see Reactions 10-17 in Table 4.1) which constitute 4 signaling cycle motifs (e.g., Reactions 10 and 11 forms a signaling cycle: $\mathrm{MCIP} \underset{\mathrm{CaNc^*}}{\overset{\mathrm{BMK1}}{\rightleftharpoons}} \mathrm{MCIPp}$. Similarly, three other signaling cycles are formed by Reactions 12 and 13, Reactions 14 and 15, Reactions 16 and 17, respectively).

Although several modeling studies have been previously published about the properties of the signaling cycle motif [83,84], it is rarely noticed that in many cases, the appearance of signaling cycle is accompanied with an inhibitor as described in Fig. 6.4a. A good example (as described in Fig. 6.4b) is that protein 14-3-3 inhibits the NFAT signaling cycle formed by Reactions 14 and 15 in Table 4.1. Another example found in the signaling network shown in Fig. 4.1 is that protein 14-3-3 inhibits the $MCIP^{PP}$ signaling cycle formed by Reactions 12 and 13 in Table 4.1. What we are particularly interested in is how the accompanying inhibitor will modulate the properties of the signaling cycle motif.

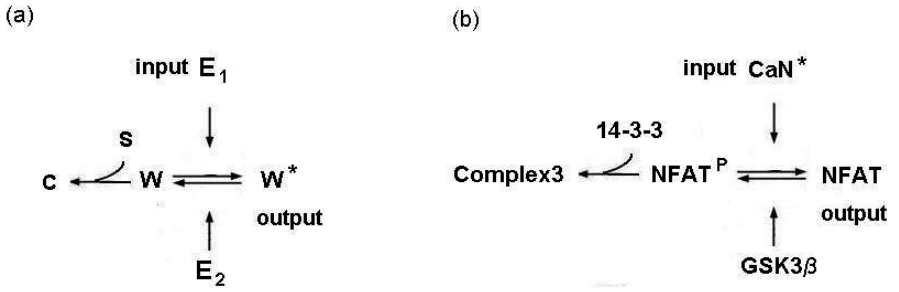


Figure 6.4. Signaling cycle with an inhibitor. (a). In signaling cycle motif, Enzyme E_1 activates protein W (to produce W^* which is the active form of W) and E_2 deactivates it. The inhibitor S binds with W to form a complex C which can not be activated by E_1. (b) An example of signaling cycle with an inhibitor found in Fig. 4.1. Here, protein 14-3-3 acts as the inhibitor.

The signaling cycle with an inhibitor can be represented as a set of three reactions in Cellerator form as follows:

$$\left\{\left\{W \overset{E_1}{\rightleftarrows} W^*,\ k_1,\ k_{-1},\ k_2\right\},\ \left\{W^* \overset{E_2}{\rightleftarrows} W,\ k_3,\ k_{-3},\ k_4\right\},\ \{S + W \rightleftarrows C,\ k_5,\ k_{-5}\}\right\}$$

The ordinary differential equations used for simulating the signaling cycle with an inhibitor are generated automatically using Cellerator. The detailed 8 equations are:

$$\frac{dC(t)}{dt} = -k_{-5}C(t) + k_5 S(t)W(t)$$

$$\frac{dE_1(t)}{dt} = -k_1 E_1(t)W(t) + k_2 W \cup E_1(t) + k_{-1} W \cup E_1(t)$$

$$\frac{dE_2(t)}{dt} = -k_3 E_2(t)W^*(t) + k_4 W^* \cup E_2(t) + k_{-3} W^* \cup E_2(t)$$

$$\frac{dS(t)}{dt} = k_{-5}C(t) - k_5 S(t)W(t)$$

$$\frac{dW(t)}{dt} = k_{-5}C(t) - k_5 S(t)W(t) - k_1 E_1(t)W(t) + k_4 W^* \cup E_2(t) + k_{-1} W \cup E_1(t)$$

$$\frac{dW^*(t)}{dt} = -k_3 E_2(t)W^*(t) + k_{-3} W^* \cup E_2(t) + k_2 W \cup E_1(t)$$

$$\frac{dW^* \cup E_2(t)}{dt} = k_3 E_2(t)W^*(t) - k_4 W^* \cup E_2(t) - k_{-3} W^* \cup E_2(t)$$

$$\frac{dW \cup E_1(t)}{dt} = k_1 E_1(t)W(t) - k_2 W \cup E_1(t) - k_{-1} W \cup E_1(t)$$

Where $C(t), S(t), W(t), W^*(t), E_1(t)$ and $E_2(t)$ denote the concentrations of species C, S, W, W^*, E_1 and E_2 respectively. $W \cup E_1(t)$ denotes the concentration of the intermediate complex formed by W and E_1. $W^* \cup E_2(t)$ denotes the concentration of the intermediate complex formed by W^* and E_2. $k_1, k_{-1}, k_2, k_3, k_{-3}, k_4, k_5, k_{-5}$ are rate parameters.

We numerically solve the above equations for various values of the total concentrations of the inhibitor (i.e., S^{tot}) and depict the steady state concentration of W^* (i.e., W^{*ss}) as a function of the total concentration of E_1 (i.e., E_1^{tot}) as shown in Fig. 6.5a.

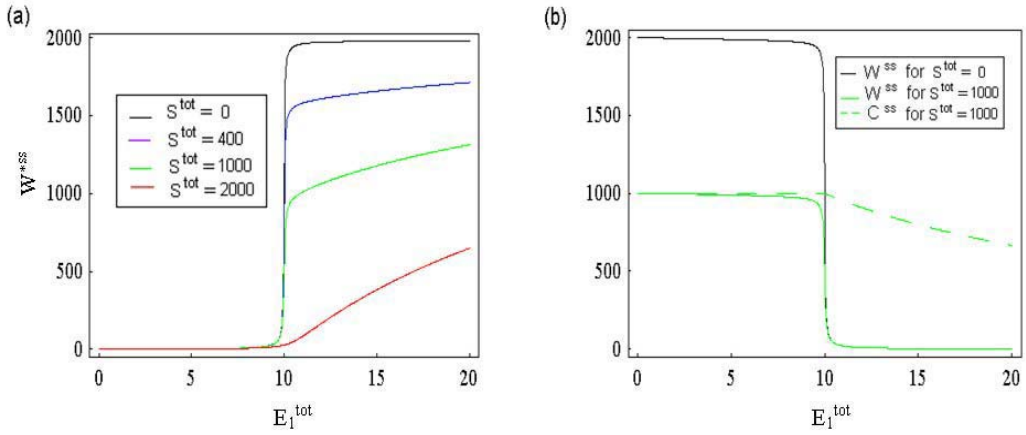


Figure 6.5. Modulation on the steady-state response of the signaling cycle by an inhibitor. (a) The black, blue, green and red curves describe the steady-state response curves

(W^{*ss} as a function of E_1^{tot}) for $S^{tot} = 0,400,1000,2000$, respectively. The rest parameter values are as follows: $k_1 = k_{-1} = k_2 = k_3 = k_{-3} = k_4 = k_5 = k_{-5} = 1, E_2^{tot} = 10, W^{tot} = 2000$. W^{tot} denotes the total concentration of W. In the simulations, the following initial conditions are used: $E_1(0) = E_1^{tot}, E_2(0) = E_2^{tot}, S(0) = S^{tot}, W(0) = W^{tot}$ and the initial values of all the rest variables are 0. (b) The black and green solid curves describe the steady-state concentration of W (i.e., W^{ss}) as a function of E_1^{tot} for $S^{tot} = 0$ and $S^{tot} = 1000$, respectively. The dashed green line describes the steady-state concentration of C (i.e., C^{ss}) as a function of E_1^{tot} for $S^{tot} = 1000$.

From Fig. 6.5a, we can see that for various values of S^{tot}, W^{*ss} hardly increases (from 0) when $E_1^{tot} < 9$. For $S^{tot} = 0$, W^{*ss} rises to less than 200 when E_1^{tot} increases from 9 to 9.9, then increases extreme rapidly to more than 1800 when E_1^{tot} increases from 9.9 to 10.1 and finally rises very gradually in a hyperbolic way to approximate its limit value (i.e., 2000) when E_1^{tot} increases from 10.1 to 20. We name the narrow range of E_1^{tot} ($9.9 \le E_1^{tot} \le 10.1$) as the critical region. For $S^{tot} = 400$, the end value of W^{*ss} during this critical region is around 1500, which is lower than that for $S^{tot} = 0$. For $S^{tot} = 1000$, the end value of W^{*ss} during this critical region is even lower (less than 1000) whereas for $S^{tot} = 2000$, there is little change of W^{*ss} during this critical region.

From Fig. 6.5b, we can see that for the signaling cycle without inhibitor (i.e., $S^{tot} = 0$, the black curve), the steady-state profile of W^{ss} is almost mirror symmetrical to that of W^{*ss} (i.e., the black curve in Fig. 6.5a) along the horizontal line $W^{*ss} = 1000$. This is because in this case, $W(t) + W^*(t) \approx W^{tot} = 2000$ (Please note that $W(t) + W^*(t) + W \cup E_1(t) + W^* \cup E_2(t) = W^{tot} = 2000$ and $W \cup E_1(t) + W^* \cup E_2(t) \le E_1^{tot} + E_2^{tot} \le 30$). For the signaling cycle with an inhibitor (i.e., $S^{tot} = 1000$, the green curve in Fig. 6.5b), when E_1^{tot}<9, both W^{ss} and C^{ss} are around 1000. Then W^{ss} extreme rapidly decreases to almost 0 during the critical region whereas C^{ss} gradually decreases when E_1^{tot} increases from 9.9 to 20 and the decreasing rate of C^{ss} is almost equal to the increasing rate of W^{*ss} shown in Fig. 6.5a (the green curve) because in this case, $W(t) + W^*(t) + C(t) \approx W^{tot} = 2000$.

6.3 Discussion

As we can see from Section 6.2.1, several kinds of network motifs such as autoregulation motif, SIM motif, feedback loop motif and coupled feedback loop motifs do exist widely in metal ion homeostasis and signaling systems. It is interesting to notice that relatively simple network motifs can be embedded in more complex network motifs, for instance, the coupled NN motif shown in Fig. 6.2d contains a FFL motif ($[Zn^{2+}]_i$ regulates Zrt1 both directly and indirectly). The coupled NN motif shown in Fig. 6.3a contains a SIM motif ($[Zn^{2+}]_i$ regulates both Zur and ZntR).

The great number of negative feedback loops shown in Fig. 6.2b-d and Fig. 6.3 confirms our previous statement that negative feedback controls constitute the central scheme for maintaining homeostasis. The various strategies used by biological cells for maintaining homeostasis as discussed in Section 1.3.3.2 and 1.3.3.3 can be represented in the framework of network motifs (especially feedback loop motifs). Thus investigations of the properties of the network motifs are quite important in order to gain insight into the general designing principle of metal ion homeostasis and signaling systems.

The most well-known characteristic of signaling cycle is that the steady-state response of this basic cycle can be in a highly sigmoidal (ultrasensitive) regime as can be clear seen in the behavior of the black curve in Fig. 6.5a during the critical region [83,84]. Inhibitor S can modulate the steady-state response of the signaling cycle by suppressing the amplitude of the value change of W^{*ss} during the critical region and increasing the rate of the value change of W^{*ss} after the critical region (see Fig. 6.5a). The investigations shown here can help us understand why signaling cycle can function as the pivot where the interaction of multiple signaling pathways happens (e.g., the interaction between 14-3-3-NFAT pathway and calcineurin-NFAT pathway takes place through the signaling cycle shown in Fig. 6.4b. The interaction between the BMK1/ERK5[26] signaling pathway and calcineurin-MCIP pathway happens through the $MCIP^P$ signaling cycle formed by Reactions 10 and 11 in Table 4.1). It is known that three simple systems (activator-inhibitor system, substrate-deplete system and delayed negative feedback system) containing a signaling cycle can generate oscillating behavior [213]. We will further investigate the modulating effect on the oscillating behavior of these systems after adding an inhibitor to the signaling cycle in these systems.

In this Chapter, we gave a concise summary of the functions of various network motifs in cellular networks and enumerated those found in metal ion hoemoestasis and signaling systems. Particularly, we used simulation results of an ODE model to show the modulation effect on the properties of the signaling cycle motif by an inhibitor. In Chappter 7, we will summarize the thesis, do some final discussions and indicate the future work.

[26] ERK5 is a synonym of BMK1 [95].