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Hypothesis

Conception of biological networks at the molecular level as orchestrated systems of oscillators representing interconnected modular molecular clocks

Alexander Belostotsky

Vavilov Institute of General Genetics, Moscow, Russia. E-mail: alexbel.system@gmail.com.

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Biological networks are extremely complex objects. Still representation of them remains to be very simple. Here hypothesis representing all regulatory cellular processes as cycles connected to each other is provided. Cycles were introduced as minimal parts of network containing negative feedback loop. All cycles were interconnected between each other by common members of network; it can be gene, protein or metabolite. Gene networks, metabolic networks and signaling cascades were represented as cycles with different characteristic period of oscillations. Dynamical connectivity takes place according to the period of oscillations. The period of high frequency oscillation must be "drawn" into the period of low frequency oscillation at least to avoid destructive interference of oscillations and moreover to maintain low frequency process that is supposed to be circadian rhythm and presumably cell cycle. So, corresponding multi-level model of regulatory networks as a composition of cycles which function as complex modular molecular clocks represented. This model shed light on biological networks at molecular level organizations and dynamics.

Key words: Gene network, metabolic network, signaling networks, negative feedback loop, oscillations, molecular clock, circadian rhythm.

INTRODUCTION

Insights from biological network structure and oscillations

All known regulatory networks contain huge amount of negative feedback loops. It is separate question why those loops are underestimated in pathways schema but it is quite clear that their role in metabolism and its regulation is enormous. Metabolic, gene and signaling networks creating one super-network are networks that really contain negative loops that really matters if we focus on the dynamics of the processes.

Negative feedback loops are crucial for network dynamics; they cause two different process dynamics pattern: one single peak or oscillations. These regimes are different but they both really can correspond to actual oscillatory pattern. First of all, the stronger the negative loop is, the more possible oscillations take place and the more frequent oscillations are (Cheong and Levchenko, 2010). So, single peak can be initial step leading to oscillations. Moreover, "single peak" variant was generally studied in a quite good manner. It was due to good correspondence with interest of medicine – just fix one relevant effect. No matter if this effect is repeated later during an experiment, it is interesting that it takes place. "Oscillations" variant is more sophisticated one. It takes into account the dynamics of the process in a broad range of time. That is why just one peak principally can not be true alternative variant to oscillatory behavior – because it is estimated in less strict conditions and so it can be just one peak in series of peaks creating complex spectrum, for example oscillatory one. So, oscillations are more general and precise variant of regulatory network dynamics.

Insights from biological networks and oscillations

It would be strange if oscillatory mechanisms in natural networks were not ubiquitous. Oscillations in metabolic, signaling and gene networks were observed

experimentally in great studies. Oscillations in the center of all cellular metabolism, glycolysis, are well known (Berridge and Rapp, 1979). These oscillations can be the cause of oscillations in other metabolism or at least in metabolic pathways tightly interconnected with it. Examples of metabolic oscillations connected to glycolisis can be also seen in purine nucleotide synthesis pathway (Tornheim and Lowenstein, 1974).

Oscillations in signaling networks were detected in case of famous NFkappaB signaling (Lee and Covert, 2010). In this case, periodic activity of kinases in pathway leads to periodic activity transcription factor coupled with its translocation from cytoplasm into nucleus and from nucleus to cytoplasm. IkB, being inhibitory subunit of NFkB, mediates negative feedback loop, which leads to such oscillations. Many components of pathway cascade, actively studied proteins appear to oscillate with equal period (Sun et al., 2008). It appears that some "wave" of activation propagate through cascade members. Later "wave" propagates after some particular characteristic time passes, and so on while cascade is active.

Oscillations in gene networks are tightly connected to oscillations in signaling cascades which regulate gene expression. Such oscillations were detected in case of genes expression oscillations regulated by such “oscillating” signaling including already mentioned well studied NFkB (Paszek et al., 2010). Oscillating transcription factor activity leads to oscillating genes transcription level. It is also crucial that oscillations induced by just one factor are very widespread in cell. For example, TNFalpha induces oscillations of more than 5000 genes through NFkappaB (Sun et al., 2008).

The best studied oscillation regime maintaining gene network is circadian rhythm gene network, in which oscillations of particular proteins activity and particular gene expression occur with period of 24 h. Such rhythm seems to be found in all phyla of life (Merrow et al., 2005). Circadian rhythm was found to be associated with activity of special proteins (Wilsbacher and Takahashi, 1998), coding by genes called circadian clock genes. These proteins are functionally connected with each other in such a way that they regulate each other by negative feedback loops that allows oscillatory dynamics mode. Research of circadian clock genes revealed that they regulate many processes including even pathogen response genes (Sherman and Froy, 2008; McClung, 2011) and also play role in cancer (Ko and Takahashi, 2006). It was clearly reported that circadian clock is closely connected to other cellular processes like metabolism, transcription regulation and cell cycle (Yamada and Forger, 2010) that means that circadian clock genes are likely to be linked in gene network with genes coding proteins involved in these processes.

Circadian clock itself really seems to be functionally connected to cell cycle regulations (Hunt and Sassone-Corsi, 2007), so cell cycle can be a cycle regulated by other cycles including circadian clock. Testing this hypothesis is a separate task but we can assume that cycles maintaining circadian rhythm maintain cell cycle as well.

HYPOTHESIS AND MODEL

Cyclic structure of networks

Oscillations of regulators activity and regulated genes expression occur in minutes, circadian rhythm occur in hours but could it be some connection between signaling and metabolic events, induced gene expression and circadian rhythm?

From modeling and experimental data mentioned in introduction we can assume that oscillatory mechanisms are quite usual for cell. They occur in different metabolism pathways, signaling pathways and gene networks, all of which are actually networks. But all networks are surely closely interconnected and it would be nice to see the more general sheme of oscillations. Could these oscillations in different cycles create one general picture?

First of all, new consideration needs new representation. It would be right to use knowledge about cycle structure in gene, signaling and metabolic networks in networks and pathways drawing. So it is an adequate way to represent them not as one-way-directed lines, but as cycles connected to each other by particular nodes. In this way, the network is created, network consisting of cycle graphs. The simplest network is represented on Figure 1. Cycles of different level correspond to cycles with different period of oscillations. Inner cycle relates to the highest frequency oscillations, intermediate cycle relates to the middle ones and outer cycle relates to the lowest frequency oscillations. Cycles of different levels are connected with each other by particular members of cycles. In this way, it is crucial that one cycle influences another one. Oscillatory dynamics in this network can be modeled by composition of oscillatory dynamics in subnetworks.

This representation has very important feature: it does not change much upon progress of new members and relations detection. This is scheme depending only upon period of oscillations and some particular reference members of network forming cycles.

It is quite clear that networks representing examining gene networks or regulatory pathways must have much more members in each cycle, much more cycles of each level, connected with each other and much more cycles within each level in terms of "inner", "intermediate" and "outer". All these cycles are connected with each other by particular nodes. Abstract example of such network is depicted on Figure 2. Inner cycles are presumably composed primary from metabolism-driving enzymes, intermediate ones are built up from members of signaling cascades and genes regulated by them, some

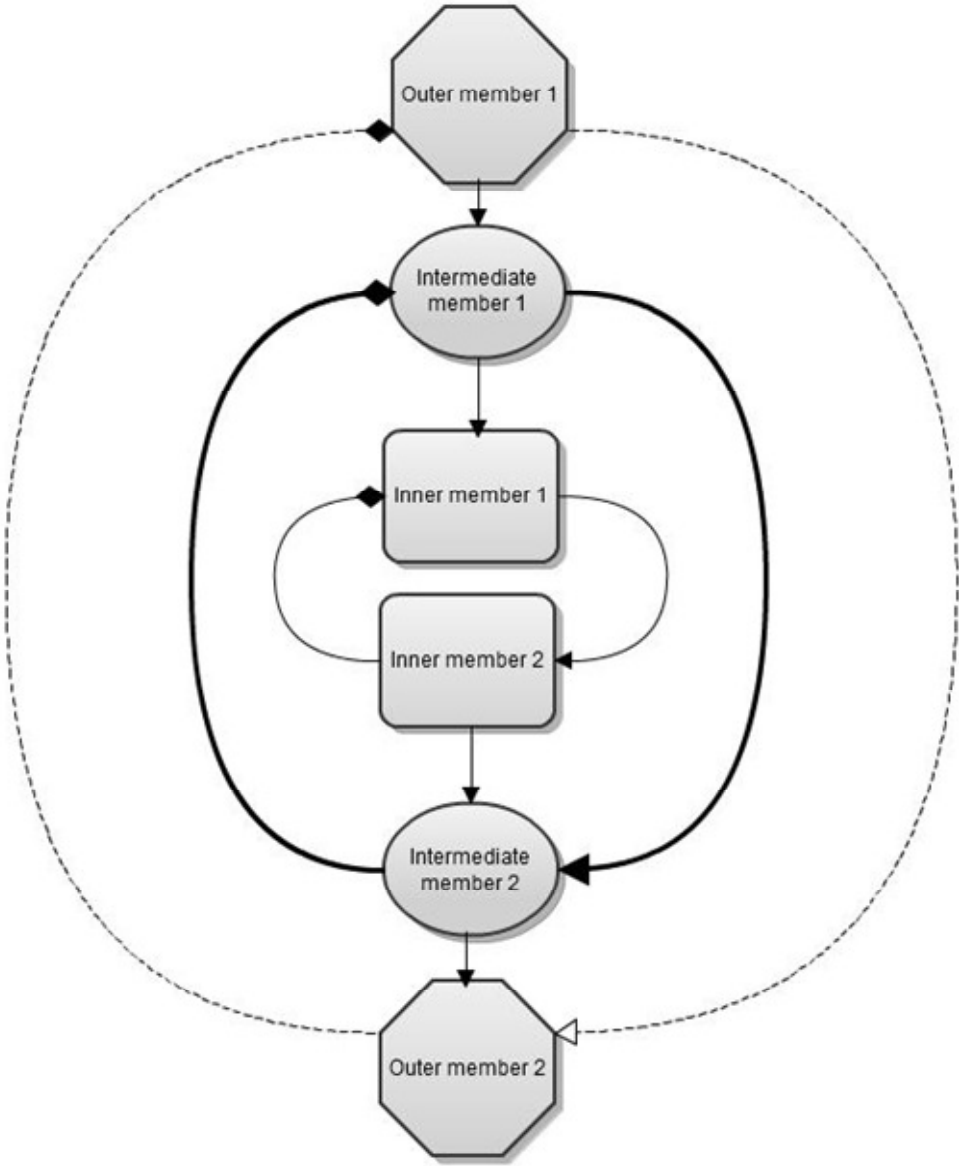

Figure 1. The simplest model of cycle-represented network. Inner members are members of inner cycle, which is cycle with high frequency oscillations, intermediate ones are members of intermediate cycle, which is the cycle with intermediate frequency and outer members are members of outer cycle, cycle with low frequency of oscillations. Arrows with triangle end are for activation and arrows with rhomb end are for inhibition.

autocrine loops, outer cycles are created by circadian rhythm maintaining proteins and it can be also cell cycle regulators.

Cycles as local clocks

When cyclic structure is clearly represented we can proceed to the consideration of interconnection between signaling cascades and circadian rhythms. They seem to be tightly dynamically interconnected.

Some transcription factors are really tightly connected to circadian rhythm maintenance (Paszek et al., 2010) and the most studied case is well-known oscillating activity of NFkappaB (Marpegan et al., 2004). Taking into account that oscillations of NFkappaB activity persist for days (Lee and Covert, 2010), we can assume that this oscillation must be in some constant relations with circadian rhythm. But what to do with phase consistency? Interestingly, oscillations of signaling have quite different period but usually it is about 30, 40, "around one hour", 90 min, 3 h (Hoffmann, 2002; Nelson et al., 2004; Ashall

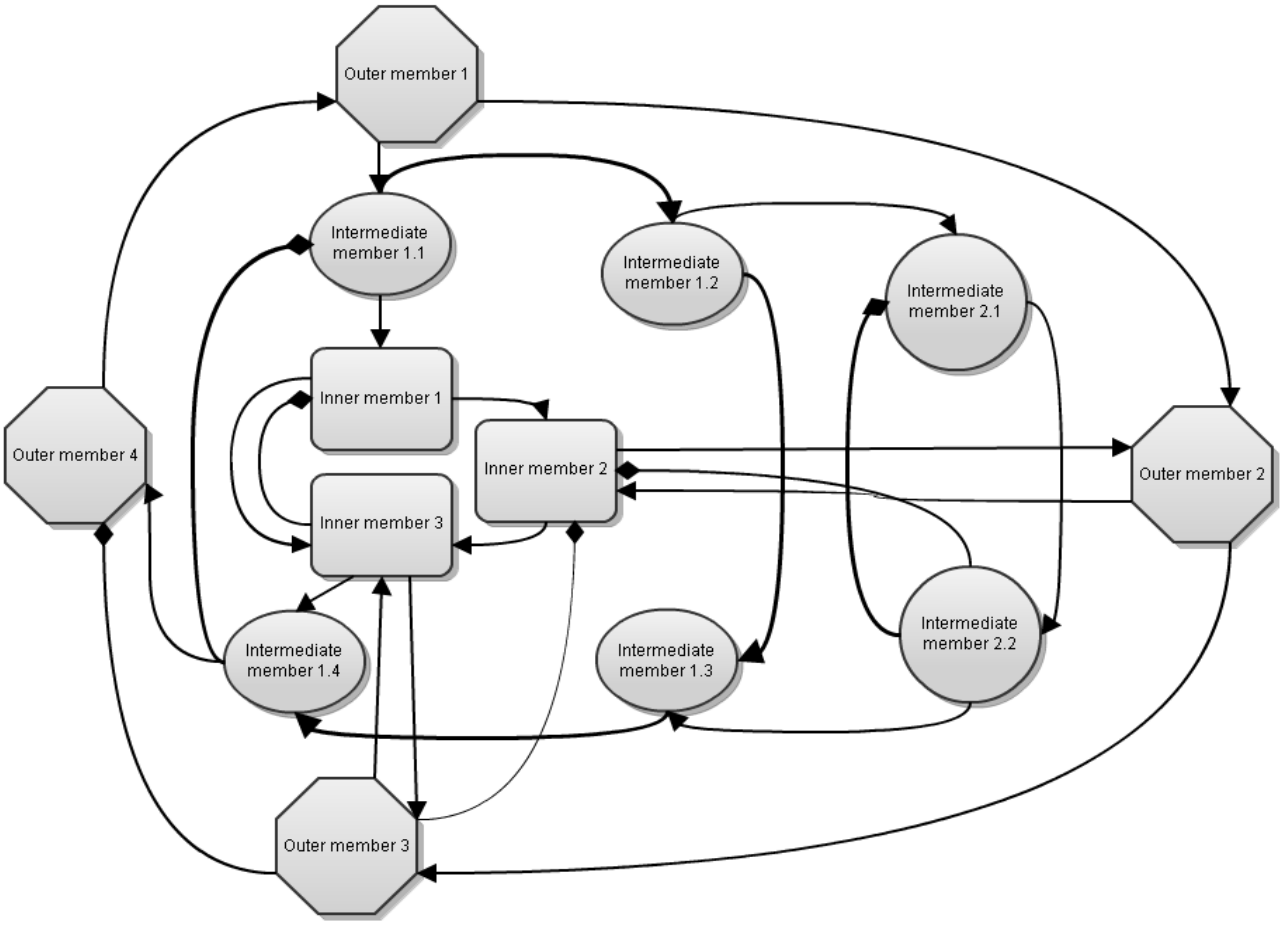

Figure 2. The more complex model of network. It is shown that there exist more than one cycle at any level. In this case there are two intermediate cycles. Thickness of arrow correlates with strength of interaction. All cycles are interconnected so study of networks demands demarcation of different cycles considering such lateral intercycle bonds.

et al., 2009). We can see that 12 and 24 h are multiple of all these numbers. It seems that at least studied signaling processes can be "drawn" into circadian clock without phase change every 24 h, so this system is quite robust during many days.

Metabolic oscillations have also quite different periods but usually this period is minutes, for example period of sustained NADH oscillation in yeast glycolys is is 1 min (Teusink et al., 1996; Berridge and Rapp, 1979), but also NADH oscillations can have period near hour detected in mouse (Voronina, 2002). Even if high frequency oscillations can affect low frequency oscillations they can be "drawn" into ones and be generally the smallest but surely very important "gear".

So, it is quite possible that oscillatory regulation of some processes coexisting with circadian rhythm is forced to be in line with circadian rhythm or even create and maintain it.

We can involve general model where each cycle is considered as gear in global clock or as local modular clock connected to global clock. All local clocks form one big clock which is considered to be circadian clock or even cell cycle.

CONCLUSION

So taking into account all points we can conclude that: (1) regulatory networks can be represented as a system of tightly interconnected cycles, (2) cycles function as oscillators at least in a broad range of conditions, (3) all oscillation are in close connection with each other both in one cycle and in different cycles, and (4) different oscillators represent modular molecular clocks connected to circadian clock and cell cycle.

To reveal the structure of these cycles and their interconnection in dynamics, robust and non-robust components, propagation of signal in such network, to make this approach real, is the goal of the next study.

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