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Quantitative understanding of transcriptional regulatory logics in modulating circadian rhythms

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Abstract

The day-to-day physiologies are largely influenced by circadian rhythms. Disruptions of such rhythms are associated with many diseases. Adjusting them to a healthy one can be promising to treat different circadian rhythms disruption associated diseases such as sleep disorders, cardiovascular disorders, metabolic disorders. The regulations underlying the circadian rhythms are much complicated and systematic which may involve thousands of genes. In mammals, these robust circadian rhythms are primarily intended by the concerted molecular interplays, knowingly, transcriptional-translational feedback loops (TTFLs). The collaborative interactions among a large number of genes intend to sustain the TTFLs. It facilitates to generate the primary transcriptional oscillations among the clock genes and genome-wide rhythmic oscillations. The collaborative transcriptional events act as dominant driving forces underpinning such rhythmic expressions. The mode of the transcriptional regulations depends on the concentration of the transcriptional factors (TFs) at the promoter region at a particular time point. The inclusive mechanisms of their regulations and the associated circadian rhythmic outputs across the physiology are not well defined yet. However, temporal recruitment of core-clock proteins, different transcriptional and translational regulators and chromatin modifications are imperative towards a comprehensive understanding of the spatio-temporal regulation of such complex rhythms. Despite many experimental signs of progress about the circadian transcriptional controls, there is still an interesting question remains unexplored that how do these few components belonging to the same molecular architecture are capable to govern such divergent gene expressions? Nevertheless, how they are being regulated and the landscape of their combinatorial regulatory controls have not gained any inclusive attention yet. Thus, a systematic understanding considering all-encompassing circadian TFs and their relational interplay could help us to decode the intricated transcriptional regulatory logics composed by different TFs. Such comprehensive

understanding may lead to unleashing their potential to therapeutically modulate the circadian rhythms. Experiments alone are indeed quite challenging to achieve this. Decoding the inclusive transcriptional insights along with multifaceted molecular regulations remained out of reach with prevailing approaches. They are limited by the complexities of more integrative algorithms that accommodate different layers of molecular information quantitatively into a single framework.

Studies indicated the knockout of the circadian TFs results in changing the rhythms. And, rescuing them helps to regain the circadian functionality substantially. However, knocking out all possible combinations of circadian TF-genes experimentally is merely very tedious, time-consuming and expensive. And, some essential genes cannot be knocked out. The magnitude of disruption of the circadian rhythmic fluctuations may also vary in disease conditions and even from individual to individual. These are serious concerns which were weakly understood. Due to the lack of advanced quantitative approach, these have remained a great challenge with traditional practices for reversing the disrupted circadian rhythms. Another level of challenge is not only aligning the rhythms but also, having a strong understanding of the directionality of the alignment varying in different clinical contexts is the most crucial. Consequently, a thorough quantitative understanding at the molecular level of the clock control mechanisms is essential. To address these ambiguities, a quantitative understanding of the circadian gene regulation and the molecular interplay among the key regulators are quite important.

An alternative yet the operative approach is the reconstruction of transcriptional network with those genes having circadian fluctuations by computational simulations. It may capture a systematic snapshot of such gene regulation network at a dynamic scale. Inferring them is again a complicated task as the large numbers of variables are unknown in the systems. There is also a lack of tools to capture and integrate the dynamic view which is biologically relevant. Virtual knockout experiments leverage

in inferring such dynamic transcriptional regulatory networks iteratively and effectively. The molecular machinery underpinning the circadian rhythms possess high-temporal resolutions. Thus, it is also quite challenging to construct the network of those genes under the influence of TTFLs at dynamic scale using existing methods. Most of the prevailing approaches are quite limited by the quantitative understanding of the transcriptional landscape thoroughly.

Recently, one of our computational approaches, LogicTRN was proposed for modelling the transcriptional regulatory networks quantitatively. Deploying the high-resolution temporal gene expression data and the TF-DNA binding data, it calculates the TF-DNA binding occupancy, which is a quantitative estimation. It also predicts the all possible combinatorial TF-logics influencing those target genes' regulations. Here, we introduced an extended computational approach based on LogicTRN to decode the quantitative transcriptional regulatory landscapes of circadian genes. We introduced the reconstruction of quantitative transcriptional regulatory networks (qTRNs) for circadian gene regulations using LogicTRN framework. The qTRNs facilitated to discover a wide range of genes exhibiting circadian fluctuations. Their dynamic behaviours and the *cis*-regulatory logics in the networks were also estimated precisely. Based on quantitative knowledge from qTRNs, we have further developed a method for virtually knocking out the core clock component TFs to estimate the influence to perturb the circadian rhythmic fluctuations at a dynamic scale. Consecutively, the method of single/multiple genes virtual knockout was developed and used to screen the best TF/TFs combination that effectively modulates the circadian rhythmic output at a dynamic scale. They were also ordered by their influence to perturb the circadian fluctuations in the qTRNs. In future, it may indicate a way to target the molecular regulators to therapeutically modulate the circadian period lengths in a specific direction based on an individual's clinical conditions. Our results indicate the reconstruction of highly accurate quantitative regulatory networks for the transcriptional controls of the circadian gene

regulation at a dynamic scale. We have also identified the best plausible transcription factors or their combinations those can effectively modulate the circadian rhythms. Of them, the CLOCK and CRY1 double knockout preserve the highest capacity to modulate the circadian rhythm dynamics. Besides, all possible TFs/TF-combinations were ordered in terms of their capacity to influence the qTRNs at dynamic scales. Finally, our quantitative framework offers a quick, robust, and physiologically relevant way to screen and identify the most effective TFs/TF-combinations to modulate circadian rhythms. This foundation may potentially enable us to engineer the molecular regulators underpinning the circadian rhythms. This potentially indicates a clue towards adjusting the circadian rhythmic phases in desired directions depending on clinical requirements.

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