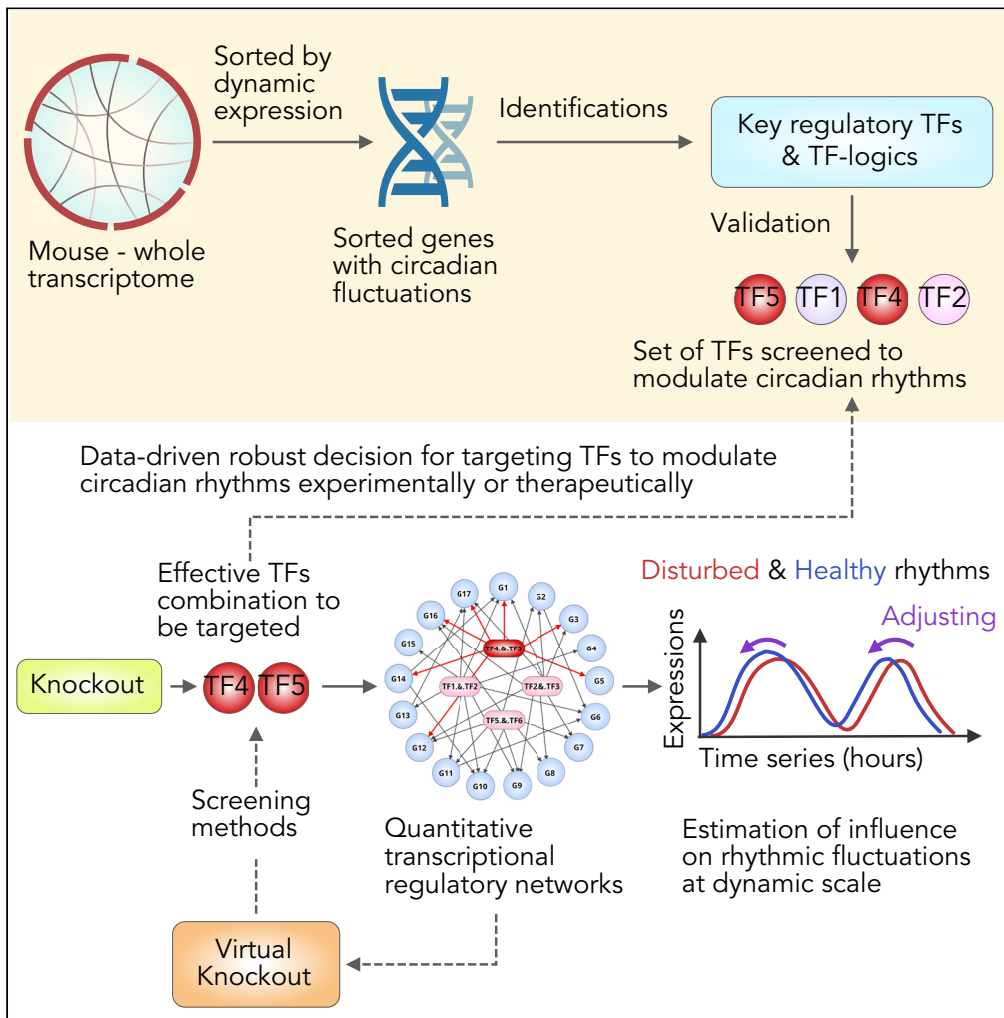**Article**

Identifying Transcription Factor Combinations to Modulate Circadian Rhythms by Leveraging Virtual Knockouts on Transcription Networks

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HIGHLIGHTS

Decoding combinatorial transcription regulation for the circadian genes using TF-logics

Precise prediction of their expression behaviors at a dynamic scale

Virtual knockout to estimate TF-combinations' influence in perturbing circadian rhythms

Potentially characterizing the druggable targets to modulate circadian rhythmic outputs

Article

Identifying Transcription Factor Combinations to Modulate Circadian Rhythms by Leveraging Virtual Knockouts on Transcription Networks

Debajyoti Chowdhury,[1,2,3] Chao Wang,[1,2,3] Aiping Lu,[1,2,*] and Hailong Zhu[1,2,4,*]

SUMMARY

The mammalian circadian systems consist of indigenous, self-sustained 24-h rhythm generators. They comprise many genes, molecules, and regulators. To decode their systematic controls, a robust computational approach was employed. It integrates transcription-factor-occupancy and time-series gene-expression data as input. The model equations were constructed and solved to determine the transcriptional regulatory logics in the mouse transcriptome network. This hypothesizes to explore the underlying mechanisms of combinatorial transcriptional regulations for circadian rhythms in mouse. We reconstructed the quantitative transcriptional-regulatory networks for circadian gene regulation at a dynamic scale. Transcriptional-simulations with virtually knocked-out mutants were performed to estimate their influence on networks. The potential transcriptional-regulators-combinations modulating the circadian rhythms were identified. Of them, CLOCK/CRY1 double knockout preserves the highest modulating capacity. Our quantitative framework offers a quick, robust, and physiologically relevant way to characterize the druggable targets to modulate the circadian rhythms at a dynamic scale effectively.

INTRODUCTION

Almost all living organisms comprise of indigenous, self-sustained 24-h rhythm generators, known as circadian rhythms. Mammalian circadian clockworks are complex and usually intended by canonical circuits comprising of many genes and a wide range of molecular regulators. They determine their rhythmic expressions and dynamic regulations over the 24 h (Koike et al., 2012; Partch et al., 2014; Ray and Reddy, 2016; Mure et al., 2018; Ray et al., 2020). The day-to-day fundamental physiological activities are significantly influenced by these circadian rhythms (Koike et al., 2012; Partch et al., 2014; Mure et al., 2018). In mammals, these robust rhythms are primarily intended by the concerted molecular interplays, knowingly, transcriptional-translational feedback loops (TTFLs) (Koike et al., 2012; Partch et al., 2014; Mure et al., 2018). Desynchrony in these rhythms are tightly associated with many negative physiological consequences and diseases (Toh et al., 2001; Son et al., 2008; Gerstner and Yin, 2010; Zee et al., 2013; Panda, 2016; Patke et al., 2017; Sutton et al., 2017).

The circadian genes under the control of TTFLs were critically involved in many fundamental biological pathways in mammalian systems (Panda, 2016). Disruptions of those genes are deeply correlated between arrhythmic productions of circadian oscillations, leading to sleep disorders, mood disorders, cognitive impairments and several other diseases including cancer, and metabolic disorders (Son et al., 2008; Gerstner and Yin, 2010; Zee et al., 2013). Progression of a neurodevelopmental disorder, Prader-Willi syndrome has been strongly linked with circadian dysfunction of a gene, *Snord116* (Hoel et al., 2016). SNORD116 is typically expressed in the brain of mice and humans (Cavaille et al., 2000; Runte et al., 2001; Galiveti et al., 2014). Experiments with deleting paternal *Snord116* indicated increased expression of several genes during the light phase including *Ube3a* (Powell et al., 2013). UBE3A targets the BMAL1 for proteasomal degradation and plays a pivotal role in maintaining the pace of clock timing at molecular level (Gossan et al., 2014). Another rare neurodevelopmental disorder, Smith-Magenis syndrome (SMS) is linked with circadian dysfunction, especially with disturbance in melatonin secretion and sleep phases (Boone et al., 2011; Goldstone et al., 2018). Study with a heterozygous deletion of *Rai1* exhibited shortened locomotor activity period linked with SMS (Walz et al., 2004; Lacaria et al., 2013).

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TFs primarily mediate the activation or repression of gene expression upon binding to the specific regulatory sequences of their target genes (TGs) (Balaji et al., 2006). Mostly they are coregulated by the influence of several TFs acting upon the promoter region of their TGs in a combinatorial manner (Claridge-Chang et al., 2001; Grundschober et al., 2001; Ueda et al., 2002; Balaji et al., 2006; Ben-Tabou de-Leon and Davidson, 2009; Lin et al., 2015; Lenstra et al., 2016; Liu et al., 2017; Liu and Tjian, 2018). Notably the transcriptional activators such as CLOCK, BMAL1, RORA, RORB, and RORC and the repressors such as PER1, PER2, CRY1, CRY2, NR1D1/REV-ERBα, and NR1D2/REV-ERBβ were found to be concertedly regulated (Mohawk et al., 2012; Jang et al., 2015; Janich et al., 2015). Their coregulated interactions with a large number of genes primarily intend to sustain the negative feedback loop, the TTFL. It facilitates to generate the primary transcriptional oscillations among the clock genes and genome-wide rhythmic oscillations (Mohawk et al., 2012). Knocking out of the major circadian transcriptional regulators form the core TTFL results in disrupting the rhythmic outputs, and rescuing them helps to regain the circadian rhythmicity to substantial extent (McCarthy et al., 2007; Ode et al., 2017; Sobel et al., 2017; Ray et al., 2020).

Adjustment of those desynchronized rhythms at the molecular level is merely a tough challenge to be resolved (Chun et al., 2014). Also, the directionality of these adjustments depending on an individual's clinical condition is crucial. The dynamic fluctuations of the transcriptional variations over the ~24 h a day have been significantly designated for determining the rhythmic behaviors in the mammalian circadian clocks (Suter et al., 2011a; Suter et al., 2011b; Li et al., 2020). This is typically achieved through recruitment and retention of the general transcriptional machinery and co-regulators that facilitate reorchestrating the local molecular environment (Koike et al., 2012; Feillet et al., 2014; Ye et al., 2014; Wollman et al., 2017; Beytebiere et al., 2019). These indicated the concerted transcriptional process as a dominant driving force underlying the gene expression rhythms (Jang et al., 2015; Janich et al., 2015). Decoding the inclusive transcriptional insights along with multifaceted molecular regulations remained out of reach with prevailing approaches (Partch et al., 2014).
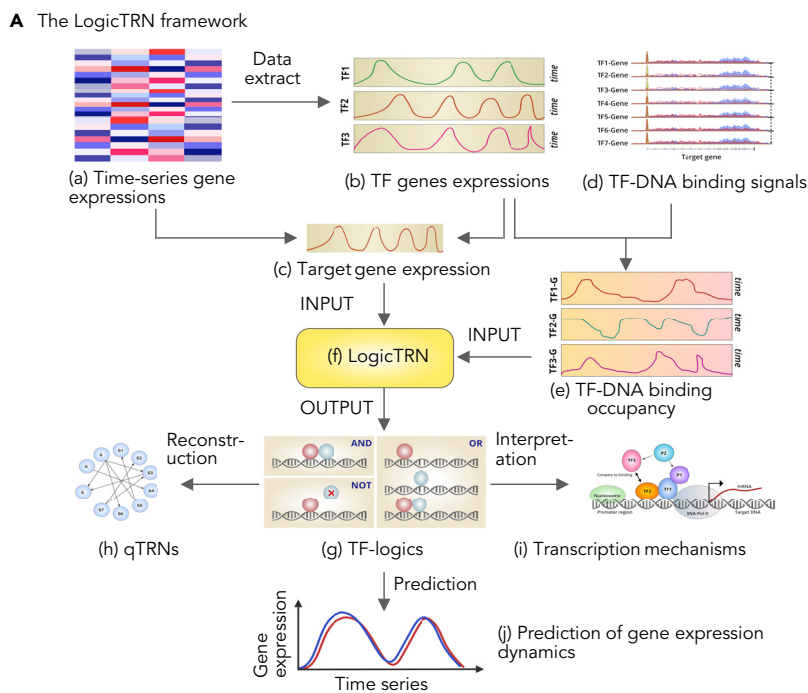
Here, we have introduced the reconstruction of quantitative transcriptional regulatory networks (qTRNs) for circadian gene regulation based on the LogicTRN framework using publicly accessible datasets (Hughes et al., 2009; Koike et al., 2012). Using these qTRNs, a wide range of genes exhibiting circadian fluctuations were well predicted with their dynamic behaviors, and the *cis*-regulatory logics (represented by "&" for AND logic, ">" for NOT logic, and "|" for OR logic) in the network were obtained. 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. It may indicate a way to target the molecular regulators to therapeutically modulate the circadian period length in a specific direction based on an individual's clinical conditions.
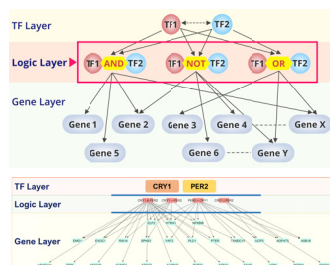
RESULTS

Here, we developed a physiologically relevant quantitative transcriptional model to decode the circadian regulation, which helps us to understand the mechanism of the clock at the molecular level. Relying on the qTRNs and the computational simulation strategy (Figure 1A), the key transcriptional regulatory factors were robustly identified including the candidate TFs and their combinatorial interplays toward regulations (Figure 1B). It was also found that the key factors could effectively modulate the circadian rhythms to exert its influence at a dynamic scale.

Reconstruction of qTRNs to Decode the Regulatory TF-Logics for the Genes with Circadian Fluctuations
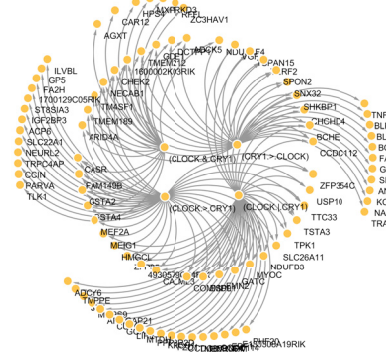
qTRNs serves as an essential tool to elucidate the underlying transcriptional regulatory networks among the core TFs. To identify the dynamic combinatorial interactions of the circadian TFs to their target genes belonging to the qTRNs, we used mouse liver whole-tissue transcriptome microarray to measure the circadian gene expression (Hughes et al., 2009). Then, the TF-DNA binding occupancy was quantitatively estimated integrating the whole liver gene expression data (Hughes et al., 2009) and high-throughput TF-DNA-binding signals (Koike et al., 2012). It helped us to predict the plausible TF-logics responsible for transcriptional regulations comprising in the qTRNs and to predict their dynamic behavior successively. Based on the wide range of well-predicted genes using those 10 core circadian TFs, the qTRNs were reconstructed. The reconstructed qTRNs included the total number of 929 TGs. The network analysis identified

A The LogicTRN framework

(a) Time-series gene expressions

(b) TF genes expressions

(d) TF-DNA binding signals

(c) Target gene expression

(f) LogicTRN

(e) TF-DNA binding occupancy

(h) qTRNs

(g) TF-logics

(i) Transcription mechanisms

(j) Prediction of gene expression dynamics

B TF-logics and logic layer

C The sub-network of the qTRN

Figure 1. The LogicTRN Framework, Logic Layers, qTRNs, and Prediction of Dynamic Gene Expression Behaviors

(A) The schematic representation of the integrated computational framework based on LogicTRN. It is an approach for constructing transcriptional regulatory networks and characterizing the TF-logics for the genes with having circadian fluctuations.

(B) A representation of the logic layer in transcriptional regulation (upper) with an example of the logic layer formed by the CRY1 and PER2 and their corresponding TF-logics while regulating their downstream genes (lower). In this example, CRY2 and PER1 were found to constitute four different types of regulatory TF-logics combinations—(CRY1.&.PER2), (CRY1.>.PER2), (PER2.>.CRY1), and (CRY1.|.PER2)—to regulate the downstream expressions of 26 genes. This additional logic layer provides a quantitative description of the gene regulation and regulatory controls.

(C) The subnetwork comprising of CLOCK and CRY1 TFs and associated TF-logics combination and their networks of TGs. In this subnetwork, CLOCK and CRY1 act as the key candidate regulatory TFs to exhibit their transcriptional regulatory controls by constituting four different forms of TF-logics—(CLOCK.&.CRY1), (CRY1.>.CLOCL), (CLOCK.>.CRY1) and (CLOCK.|.CRY1) —to regulate their TGs. These four TF-logics function as a hub in the qTRN and each logic acts distinctly to control distinct group of genes.

several circadian TFs, CRY1, CRY2, PER1, PER2, and CLOCK to be dominant in the circadian transcriptional regulations. They used to regulate the major number of TGs that are likely to play various functions in generating circadian rhythmic outputs in abundance (Figure 1C). Those TFs were also used to interact with several other genes that were not predicted to have circadian fluctuations with our analysis. Of

them, CRY1 and CRY2 were found to regulate a total number of 638 genes out of a total of 929 genes in the entire network. The qTRNs showed that the CRY1 (n = 45), CRY2 (n = 71), and their combinations of logics, including CRY1.&.CRY2; CRY1.|.CRY2; CRY1.>.CRY2; and CRY2.>.CRY1 (n = 74) had the most crucial regulatory influence over their TGs' transcriptional regulations (where n indicated the number of the TGs regulated). Rest of the TGs (n = 448) were found to be regulated by the CRY1- and/or CRY2-associated combinations with other 8 TFs. A sub-network, comprising of different combinations of CRY1 and CREBBP, was found as well (Figure 1C). This analysis potentially introduced the concept of TF-logics in the circadian transcriptional landscape. It is an additional layer between the circadian TFs and their TGs in their transcriptional regulations (Figure 1B).

A part of the well-characterized gene list has been considered to explain the consistency of our predictions and the supporting evidence from experiments. A set of the TGs, *Alkbh27, Ebpl, Myo1b, Rab32, Sumo3, Abhd14a, Asb13, Fahd1, Apoc3,* were found to be regulated by the (CRY1.&.PER2) TF-logic. They were also found to share similar dynamic profiles as well. Three genes, *Apoc3, Fahd1,* and *Sumo3,* out of those nine genes having predicted circadian fluctuations at dynamic scales, were experimentally reported to have circadian-like features.

Transcriptional Regulatory Logics Essentially Interpret the Mechanisms of Circadian Gene Regulations
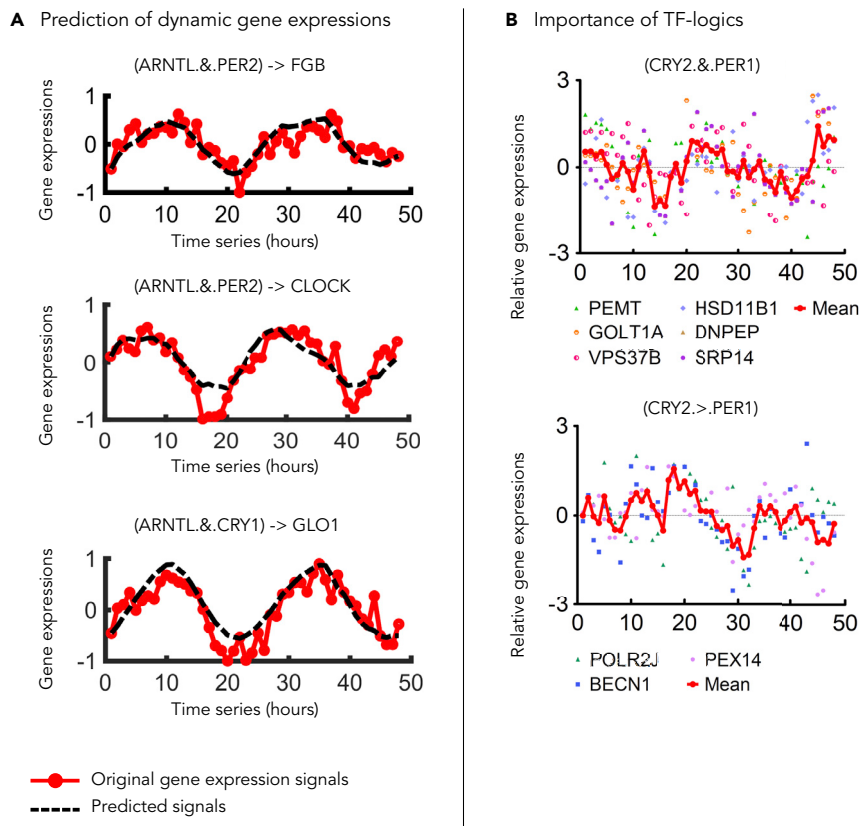
Characterization of genes with circadian fluctuations with their transcriptional regulatory logics implies their expression behaviors at a dynamic scale. We considered the time-dependent dynamic interactions among different transcriptional regulators across genome-wide. Comprehensive analysis of these transcriptional events across the entire mouse liver genome over 24 h was evaluated using 10 core circadian transcriptional regulators, CLOCK, BMAL1, CRY1, CRY2, PER1, PER2, NPAS2, EP300, CREBBP, and POLR2A (Koike et al., 2012).

The dynamic profiles of the genes belonging to the qTRNs were predicted. The very well-predicted dynamic profiles wherein the dynamic predictions are well matching with the original gene expression profiles have cyclical expression patterns over the 24 h. A total of 461 genes with circadian fluctuations were characterized (Table S1). Altogether those 461 genes were characterized to have rhythmic fluctuations and most likely to exert influence in various circadian outputs. Those genes were clustered into three groups (Figures S1–S3). These genes were further functionally characterized cluster wise using Enrichr program to find the maximum enrichment for OMIM disease information and to have the most likely pathways using KEGG pathway analysis (Chen et al., 2013; Kuleshov et al., 2016).

The genes belonging in the cluster one (n = 160) have been linked with having maximum enrichment for cholestasis, a decreased biliary flow, cardiomyopathic syndromes, and several types of cancers including, squamous cell carcinoma, adenocarcinoma, melanoma, and pancreatic cancer. The KEGG pathway analysis indicated the most enriched pathways related to those genes are AMPK signaling, cholesterol and fatty acid metabolism, and necroptosis pathways (Figure S1).

The genes (n = 173) belonging to the cluster two showed maximum enrichment for several neurodegenerations, thrombophilia among the coagulative cardiovascular diseases, and leukemia (Figure S2). Few genes from this cluster have been well characterized to exert similar disease profiles with experimental supports. For example, *Spon2* gene has been established to exert significant roles in developing cardiovascular diseases and were well characterized as possessing the tight circadian rhythmic fluctuations in its expressions. The KEGG analysis supports the OMIM disease enrichment based on this cluster by indicating the most likely terms associated with this cluster of genes includes the SNARE interactions in vesicular supports, cholesterol, sphingolipids, and fatty acid metabolisms. The gene *Apoc3* has been strongly endorsed with cholesterol and fatty acid metabolism pathways and also been demonstrated with several experiments (Solt et al., 2011; Ma et al., 2015; Reinke and Asher, 2016).

The genes (n = 128) in the cluster three exhibited maximum enrichment for diabetes, myocardial infarction, obesity, and glycogen storage diseases (Figure S3). Few genes, *Gys1, Ucp3, Mbl2,* from these clusters are most relevant to developing those diseases. And, they showed most likely to impart in the metabolism of xenobiotics, coagulation cascades, and ribosome transports at p value < 0.005. From cluster three, several genes, *Apoc1, Pemt, Maob, Pex7, Tat,* have been significantly endorsed to have a link with those concerned

A Prediction of dynamic gene expressions

(ARNTL.&.PER2) -> FGB

(ARNTL.&.PER2) -> CLOCK

(ARNTL.&.CRY1) -> GLO1

—●— Original gene expression signals
- - - - Predicted signals

B Importance of TF-logics

(CRY2.&.PER1)

PEMT   HSD11B1   Mean
GOLT1A   DNPEP
VPS37B   SRP14

(CRY2.>.PER1)

POLR2J   PEX14
BECN1   Mean

Figure 2. Prediction of Dynamic Behaviors of Gene Expressions and the Importance of Logic Layers in Producing Variable Gene Expression Behaviors at Dynamic Scales

(A) The qTRN predicted dynamic gene expression profiles for the well-characterized genes with circadian fluctuations. The genes, *Fgb* and *Clock*, were exemplified here to be regulated by the TF-logic (ARNTL.&.PER2) and *Glo1* by (ARNTL.&CRY1). The dynamic behaviors of those genes expressions were considered for 48-h time series with 1-h frequency of sampling. See also Figure S4.

(B) Regulatory logics play a key role in regulating their TGs. Two TFs, CRY2 and PER1, form two different logics, CRY2.&.PER1 and CRY2.>.PER1. With each type of the TF-logic formed, they tend to regulate different sets of TGs that share almost similar dynamic profiles. Pemt, Golt1a, Vps37b, Hsd11b1, Dnpep, and Srp14 were found to be regulated by the (CRY2.&.PER1) logic. Polr2j, Becn1, and Pex14 were found to be regulated by the (CRY2.>.PER1) logic. They indicated the differential interplay of the two TF candidates, CRY2 and PER1. These two TFs formed two different logics, (CRY2.&.PER1) and (CRY2.>.PER1). It was interesting that CRY2 and PER1 formed different logics to independently regulate the different set of TGs. When they formed "AND" logic, (CRY2.&.PER1), they were found to regulate the following five TGs, Pmet, Golt1a, Vps37b, Hsd11b1, and Dnpep. On the other side, once they formed "NOT" logic, (CRY2.>.PER1), they were found to regulate another different set of TGs, Polr2j, Pex14, and Becn1. Thus, a small number of TFs are capable to form a wide range of combinations of regulatory logics. And, they tend to exert differential regulatory controls over their TGs having different signature dynamic profiles.

pathways. The connection of such genes out of the clusters, their circadian rhythmic fluctuations, and the roles in diseases are very important to be probed in depth.

We employed the LogicTRN (Yan et al., 2017) to predict their dynamic behaviors. They were well characterized to preserve the dynamic expression behaviors to maintain the circadian fluctuations in abundance over 24 h. The predicted profiles fitted the actual gene expression profiles very well (Figure 2A). This indicated firmly that the developed qTRN is biologically relevant, and it is capable enough to interpret the gene regulations mechanisms. These accurately predicted dynamic profiles also indicated several key features about the gene regulations. The group of circadian genes under the same regulatory logics exhibit similar dynamic trends in their expression profiles. The TGs, *Clock*, *Fgb*, *Fgg*, *Csrp3*, *Acsl5* and *Acox2*, were transcriptionally controlled by the same regulatory logic

(ARNTL.&.PER2) (Figures 2A and S4). These TGs under the same regulatory logic also shared a very similar and well predicted dynamic feature.

The data indicate that the genes under the control of the same regulatory logic shared similar dynamic trends. The different logics formed by the same TFs, CRY1 and PER1, were able to produce different dynamic signatures for their TGs regulations (Figure 2B). These two TFs formed two different logics: (CRY2.&.PER1) and (CRY2.>.PER1). It was interesting that CRY2 and PER1 formed different logics to independently regulate the different set of TGs. When they formed "AND" logic, (CRY2.&.PER1), they were found to regulate the following five TGs, *Pmet, Golt1a, Vps37b, Hsd11b1,* and *Dnpep* (Figure 2B). On the other side, once they formed "NOT" logic, (CRY2.>.PER1), they were found to regulate another different set of TGs, *Polr2j, Pex14,* and *Becn1* (Figure 2B). Thus, a small number of TFs are capable to form a wide range of combinations of regulatory logics. And, they tend to exert differential regulatory controls over their TGs having different signature dynamic profiles. It suggested that the TF-logic plays a crucial role in determining the fate of transcriptional regulations at a dynamic scale. Without the introduction of the TF-logic layers, such sort of fine resolution difference in transcriptional dynamics would not have been considered.

Virtually Knocked out Circadian TFs Mutants Allow to Manipulate the Iterative Interactions among the TFs and Their TGs Dynamically

Based on the qTRNs of the genes with circadian fluctuations, we developed the approach of vKO to evaluate the influence of a TF or TF-combinations in regulating the gene expressions of the genes having circadian fluctuations at dynamic scales in the absence of those concerned TFs; there will be a certain degree of discrepancies in the engagement of other TFs or their roles and recruitment fashions reflected in the network level. This was considered as a perturbed one. In vKO mutant constructions, firstly, we used LogicTRN method to predict the TF-logics responsible for driving the gene regulations (Yan et al., 2017). We generated the TF-logics for WT conditions for all those 461 well-predicted genes, those displayed circadian fluctuations in their dynamic expressions. Eventually, the TF-logics for the WT condition served as the baseline for the decision-making for TF or combination of TFs to be knocked out virtually. We have considered one to the combination of three TFs knockout conditions, as virtually single, double, and triple TFs knockouts also referred to vsKO, vdKO, and vtKO.

The results demonstrated the influence of different virtual KO mutants on individual circadian gene expression profiles with an example of a target gene *Spon2* having circadian fluctuations (Figure 3A). In WT condition, *Spon2* was regulated by the TF-logic, in the combination of (CRY1) *or* (CLOCK.|.CRY1) *or* (CRY1.>.CLOCK) logic (Figure 3A). So, as per the WT condition, *Spon2* used to get regulated by the combinatorial interactions of two candidate TFs, CRY1 and CLOCK, in different fashions. Therefore, consequently, three sets of transcriptional simulations can be performed with this target gene. Simulations with two single TF knockout, CRY1 vsKO and CLOCK vsKO, can form only one two-TF combination, thus only a condition of double TF knockout, CRY1/CLOCK vdKO. Upon vsKO of CRY1, the *Spon2* gene was still well regulated by CLOCK alone. The dynamic features of circadian rhythmicity were largely maintained (Figure 3A) with changes in the amplitude of the expressions. Again, upon vsKO of CLOCK, the *Spon2* gene was still regulated by CRY1 alone. But in this condition, the phase shifts were observed from 20th hour and 30th hour time points. In this CLOCK vsKO condition for the *Spon2* gene, approximately ~3.5 h of the phase advancement have been observed at 20th and 30th hour. And, finally, once both TFs were knocked out by CRY1/CLOCK vdKO, the gene *Spon2* was not expressed at all, thus there was no prediction of the dynamic profiles with the transcriptional simulation. Only the original gene expressions signals were present, as it was processed from the raw microarray data. After predicting the dynamic expression profiles for the gene *Spon2*, with its all possible vKO types, the predicted values for all vKO mutants and WT were extracted. Then, each transcript out of four conditions was analyzed with MetaCycle program to estimate the lag, period length, and amplitude (Wu et al., 2016). The results indicated an hour delay in period length with the CRY1 vsKO. However, with the CLOCK vsKO, it preserved the period length as similar to the WT variant at 24 h. However, the amplitude has been changed along with the dynamic gene expressions with vKO mutants. Also, the alterations of phase shifts have been noticed at the 20th hour and 30th hour as indicated and discussed earlier. The *Spon2* gene has been well supported to be regulated by the core clock-controlled regulators, including CRYs and CLOCK (Dierickx et al., 2017). It has been identified to have a vital role in cardiomyocyte and it periodically controls the calcium current (Bray et al., 2008; Dierickx et al., 2017). And, impairment of this gene is linked with altered hepatocellular metabolic functions and cardiovascular efficiencies (Bray et al., 2008).
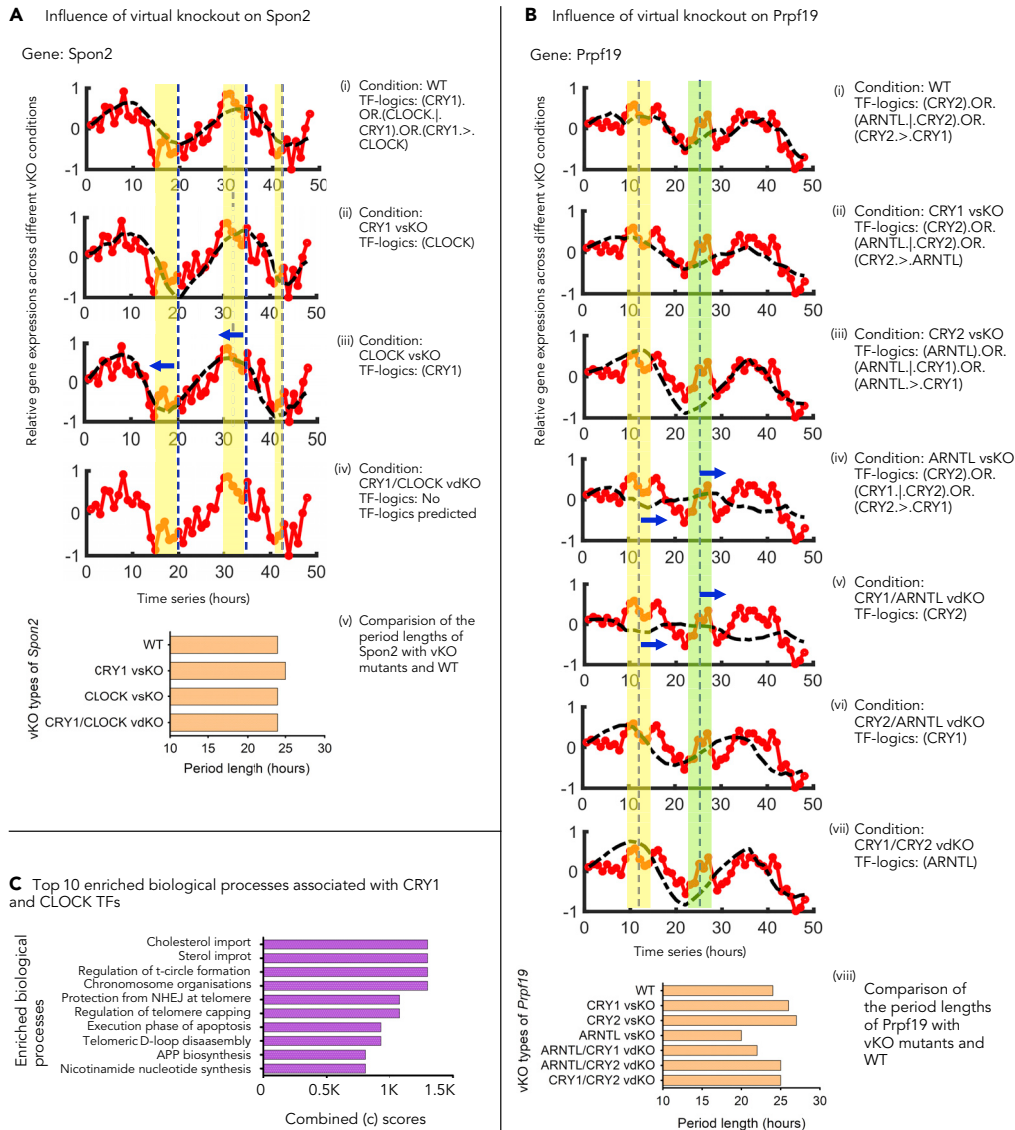
Figure 3. Influence of Virtual Knockout on Circadian Fluctuations

(A) Influence of virtual knockout in circadian fluctuations with the example of the target gene, *Spon2*. The region for the phase shifts was indicated with the yellow highlights and the directions of the shifts were indicated with the blue arrow. And, with the CRY1/CLOCK vdKO mutants, the gene, *Spon2,* was found not to be expressed. Transcriptional simulations of *Spon2* expression at WT condition (i), CRY1 vsKO condition (ii), CLOCK vsKO condition (iii) and CRY1/CLOCK vdKO condition (iv) and the comparison of their period length upon each vKO types (v). The period length was observed to be extended as 25 h in case of CRY1 vsKO mutant. However, CLOCK vsKO maintained its period length as 24 h as WT. The phase shifts were observed (highlighted in the yellow color and the direction was marked with blue arrow) from 20th hour and 30th hour time points across all the mutant transcripts. With the CLOCK vsKO condition for the *Spon2* gene, approximately ~3.5 h of the phase advancement have been observed at 20th and 30th hour (iii). Upon both the TFs knock out by constructing CRY1/CLOCK vdKO mutant, *Spon2* was not expressed at all, thus there was no prediction of the dynamic profiles with the transcriptional simulations. The original gene expressions signals were present, as it was processed from the raw microarray data (iv).

(B) Influence of different vKO mutant on a target gene, *Prpf19*. Where the region for the phase shifts was indicated with the yellow highlights and the directions of the shifts were indicated with the blue arrow. And, with the CRY1/CRY2/ARNTL vtKO mutants, the gene *Prpf19* was found not to be expressed. Transcriptional stimulation of *Prpf19* expression at WT condition (i), CRY1 vsKO condition (ii), CRY2 vsKO condition (iii), ARNTL vsKO condition (iv), CRY1/ARNTL vdKO condition (v), CRY2/ARNTL vdKO (vi), CRY1/CRY2 vdKO (vii), and their regulatory TF-logics.

(C) The top 10 most enriched biological processes associated with CLOCK and CRY1. These 10 functions are most susceptible to get perturbed upon knockout of CLOCK and CRY1 from the qTRNs. See also Table S3, and Figure S5.

Other results demonstrated the influence of different virtual KO mutants on the gene *Prpf19* having circadian fluctuations (Figure 3B). In WT condition, the target gene *Prpf19* was found to be regulated by the (CRY2) *or* (ARNTL.|.CRY2) *or* (CRY2.>.CRY1) logic, the combinations of three TFs, such as CRY1, CRY2, and ARNTL. Upon vsKO of the CRY1 TF from the WT TF-logics, the regulatory logic changes to (CRY2) *or* (ARNTL.|.CRY2) *or* (CRY2.>.ARNTL). Dynamic features of circadian rhythmicity were comparatively well maintained. Upon vsKO of the CRY2 TF from the WT TF-logics, the regulatory logic was changed, and dynamic features of the circadian rhythmicity were not conserved as WT. Upon ARNTL vsKO, and CRY1/ARNTL vdKO, the phase shifts were observed at the 10th hour time point. Again, at the 20th hour, the discrepancies were observed with the ARNTL vsKO and CRY1/ARNTL vdKO. Ultimately, taking out the three candidate TFs from qTRN using CRY1/CRY2/ARNTL vtKO transcriptional simulation, the *Prpf19* was not predicted with any TF-logics. This indicated that the target gene *Prpf19* was not expressed with the CRY1/CRY2/ARNTL vtKO condition. After predicting the dynamic expression profiles for the gene *Prpf19* with its all possible vKO types, the predicted values for all vKO mutants and WT were extracted. Then, each transcript out of seven conditions was analyzed with MetaCycle program to estimate the lag, period length, and amplitude (Wu et al., 2016). The results indicated significant changes in the period lengths in different vdKO conditions as the WT *Prpf19* exhibited 24 h as its period length over the day. But, with the ARNTL/CRY1 vdKO, a 2-h advanced phase has been observed having period length 22 h, and 1-h phase delay with 25-h period length has been identified with both the ARNTL/CRY2 vdKO and the CRY1/CRY2 vdKO conditions. However, the vsKO conditions have been turned up with quite significant changes in their period length. Three-hour phase delay was quantified with the CRY2 vsKO condition having period length 27 h a day, and 2-h phase delay has been found with CRY1 vsKO condition having the period length 26 h a day instead of 24 h as WT. It signifies that the induced vsKO and vdKO can cause significant changes in the periodicity of the expression of *Prpf19* at dynamic scale.

In vKO mutant construction process, different TFs and combination of TFs were virtually knocked out, and their influence to perturb the circadian qTRNs were computed. Based on that, the TF or TF-combinations were ranked in terms of modulating the circadian fluctuations. A total of 173 combinations were found and well characterized to influence the circadian qTRNs at dynamic scale (Table S2). Among all the ranked TF-combinations, the CLOCK-CRY1 combination was found to have the highest score. This indicated that the CLOCK-CRY1 vdKO might have the largest influence (Table S2) to perturb or modulate the circadian rhythmic fluctuations at a dynamic scale. All combinations of the TFs as per their scores have been depicted (Table S2). The subnetwork of the TGs regulated by the CLOCK and CRY1 logics was shown (Figure 1C). Among them, the influence of *Spon2* gene has been discussed in detail. Besides, the functional enrichment analysis indicated the most enriched biological processes include cholesterol and lipid metabolisms, telomere capping, and chromosomal organizations involved with the group of TGs in qTRN under the regulation of the CLOCK and CRY1 (Table S3). Therefore, it can be presumed that these TGs are the most susceptible to be altered in their dynamic expressions once the CLOCK and CRY1 will be knocked out together and those concerned biological processes may get impaired. The TGs under the control of CLOCK and CRY1 TFs and associated TF-combination were more suspected to perturb the circadian rhythms upon CLOCK/CRY1 vdKO as indicated by our study. Therefore, the circadian output and qTRN associated with these TF-combination is ought to be modulated the most. The CLOCK and CRY1 regulated qTRNs comprising most susceptible TGs comprising within the network of a rhythmic fluctuating set of genes (Figure 1C). The vKO study helped us to make further decisions to identify the rightmost TFs and/or TF-combinations to effectively perturb the associated genes expressions behavior, thus, the rhythmic circadian outputs. This was efficiently extended to screen the best plausible TFs/TF-combination to be further targeted to trigger circadian phase-shifts in the desired direction. Targeting which TFs and/or TF-combinations would be the most likely to adjust the phase shift or periodicity of the circadian fluctuations' dynamics could be efficiently determined using the aforesaid approach.

Validation of the predicted TF-logics for the genes with circadian fluctuations supports the consistency of our framework in a circadian physiological context. Our analysis validates *Apoc3* function as one of the key players to control cholesterol and bile acid metabolism rhythmically (Cho et al., 2012). Our study indicated that the *Apoc3* was regulated by the combinatorial influence of CRY1 and PER1 as forming (CRY1.&.PER1) TF-logics. Several studies established a fact that REV-ERB is actively engaged in lipid metabolism and lipid homeostasis rhythmically facilitated by expression of several crucial bile acid and lipid regulatory genes, especially *Apoc3* transcription (Cho et al., 2012). This is a major element of very-low-density lipoprotein

(VLDL). In mammals, *Apoc3* gene was found to be repressed by REV-ERBα and REV-ERBβ (Coste and Rodriguez, 2002; Raspé et al., 2002). Therefore, it indicated that the associated regulators interacting with REV-ERB are most likely to exert the impact on the expression of its target gene, including *Apoc3* (Wang et al., 2007). So, the association of *Apoc3* gene regulation and impact of REV-EBR were established. Consequentially, REV-ERBα and REV-ERBβ were found to be tightly controlled by PER2 and CRY1, which facilitate circadian expressions and control integral mechanisms to coordinate circadian rhythms and metabolisms (Cho et al., 2012). This was also strongly endorsed by the REV-ERBα and REV-ERBβ cistromic overlap. Thus, the influence of PER2 and CRY1 together forming (CRY1.&.PER2) logic to regulate the expression of *Apoc3* that exerts significant circadian metabolic functions in mammalian liver has been authenticated. Besides, the functional enrichment analysis with CRY1 vsKO condition indicated the most enriched biological process to be influenced is the bile acid secretion (Figure S5). This implied that if we took out CRY1 from the qTRNs, bile acid secretion will be significantly impaired. One of the plausible explanations backing this could be defended by the *Apoc3*-mediated impairment of the molecular clockwork. Again, with the vKO simulation with CRY1/CRY2 vdKO condition, the same gene *Apoc3* was predicted to be regulated by the combinatorial interplay of PER2 and POLR2A. They formed the TF-logic (PER2.&.POLR2A) *or* (PER2.>.POLR2A) *or* (POLR2A.>.PER2) to regulate *Apoc3* in CRY1/CRY2 vdKO mutants. It is also further validated with an experimental CRY1/CRY2 dKO mutants and found to be mostly preserved. In the experimental CRY1/CRY2 dKO mutant mice dataset, the Apoc3 was also found to be regulated by the combinatorial interplay of the same two TFs, PER2 and POLR2A, by forming the logic, (PER2.&.POLR2A) *or* (POLR2A.>.PER2). The probable reasons for such variations could be explained as the influence of other molecular regulators along with transcriptional regulatory factors. Especially, for circadian rhythmic fluctuations, there may be several other factors that are quite important in parallel with transcriptional regulations (Wong and O'Neill, 2018). Also, another potential reason for such discrepancies could be explained in terms of variations of the conditions among those datasets. The mice used in the GSE13062 dataset received restricted feeding, whereas the mice used in the GSE11923 received ad libitum diet. These dietary restrictions may influence gene expression regulations. Accessing the KO mouse experimental datasets with ad libitum diet was limited at the moment. Another further validation can be established using the example of LPIN1, which was well predicted to be regulated by the combinatorial influence of CRY1 and PER1 forming a logic, (CRY1.&.PER1) in WT condition. LPIN1 used to encode Lipin-1, which is important in triglyceride synthesis in liver and adipose tissue (Oishi et al., 2005; Zhang et al., 2008). *Lipin1* has been recognized as a circadian gene that exhibits rhythmic fluctuations in its expressions in the liver and adipose tissues (Zhang et al., 2008; Champier et al., 2012). The evidence also suggested the temporal expression pattern of *Lpin1* in liver and adipose to be strikingly high in daytime (Reue and Zhang, 2008). The *Lpin1* expression was found to be regulated by the combinatorial interplay of ARNTL and PER1 forming a logic (ARNTL.&.PER1) in the vKO simulations with CRY1/CRY2 vdKO mutant. It was found to be well consistent with the experimental CRY1/CRY2 dKO mutants, where Lpin1 was also found to be regulated by the TF logic, (ARNTL.&.PER1), in the absence of CRY1. Similarly, another gene *Pcm1* that encodes a pericentriolar material, is a component of centriolar satellites found to be regulated by CLOCK and PER1 by forming (CLOCK.&.PER1) in WT condition. Wherein, the same gene was also predicted to be regulated by the same TF-logics in the vKO mutant with CRY1/CRY2 vsKO and experimental CRY1/CRY2 dKO mutant. This predicted TF logic was quite well consistent across all the conditions. This Pcm1 gene was reported to take part in an interaction network of mRNA processing and splicing events associated with metabolic rhythms (Christou et al., 2019).

A recent finding (Ray et al., 2020) with the redundancy of *Bmal1* in maintaining daily molecular oscillations has shared a greater constancy with our claims associated with vKO approach. Ray et al. have shown that in *Bmal1* knockout mice (experimentally KO), liver tissues and skin fibroblasts exhibited circadian rhythmic oscillations at transcriptional and translational level (Ray et al., 2020). However, deletion of Bmal1 disrupts the robustness of the oscillations of the core clockwork. It was also defined that Bmal1 is important but may not be essential for all molecular oscillations. Secondly, with the experimental KO of *Bmal1*, the circadian rhythms were disrupted certainly. Wherein the altered period lengths from liver transcripts were observed ranging from 18–27 h instead of 24 h. Majority of the transcripts showed 26–27 h and fewer transcripts showed a shorter period at 18–21 h upon *Bmal1* KO. Similarly, in our study with vKO approach, we have shown that despite the physiological significance and/or direct phenotypic correlation, virtually deletion of certain key TFs from the core clock components used to disrupt the rhythmic oscillations, but in many cases, the overall dynamic patterns tend to sustain alternatively with the help of other TFs that belong to the transcriptional regulatory logic. It could be considered as an indigenous mechanism of internal

feedback systems that demands further investigations indeed. Again, with our example of *Prpf19* from the mouse liver tissue, *Arntl* or *Bmal1* vsKO was reported to show the altered period length of 20 h instead of 24 h as WT conditions. Perhaps, the transcript *Prpf19* belongs to the group of transcripts exhibiting shorter period length being disrupted upon *Bmal1−/−* as per the study indicated (Ray et al., 2020). Thus, the range of this quantification of period length fits well within the window of the experimental range of altered period length (18–21 h) with *Bmal1* KO mice.

To validate the consistency of our LogicTRN-based approach, we also performed the comparison of the performance of LogicTRN on virtually knocked out TFs as well as the dataset where the specific TFs were knocked out experimentally. The cross-dataset validation and consistency of our LogicTRN-based prediction of TF-logics for both types of datasets in contrast with WT conditions have been shown (Table S4). In this analysis, we considered two datasets GSE11923 (Hughes et al., 2009) and GSE13062 (Vollmers et al., 2009) from mouse liver. The WT mouse liver time-series gene expression dataset, GSE11923, was considered for understanding and predicting the regulatory TF-logics for WT gene expressions. This is also considered as one of our training datasets for WT condition. Successively, we have presented the predicted TF-logics at the condition of CRY1, and CRY2 TF genes were knocked out virtually to be compared with another experimental dataset, GSE13062, where *Cry1* and *Cry2* genes were experimentally knocked out (Vollmers et al., 2009). This indicated the consistency of some of the very well predicted TF-logics out of the entire analysis across those two datasets having three different conditions: one WT, one vKO, and one experimental KO with same TFs, CRY1, and CRY2. The single TF-logic-regulated target genes were predicted and quite consistent across all the datasets. And, the combination of the TF-logics was also predicted well but may not be consistent throughout in the same fashions. However, in WT condition, the candidate TFs were mostly preserved but for some of the target genes, the logics were altered. The probable reasons for such variations could be explained as the influence of other molecular regulators along with transcriptional regulatory factors.

Circadian Transcriptional Controls Are Tissue Specific

In the mammalian circadian organization, the SCN-master pacemaker presides at the top of the hierarchy (Panda et al., 2002; Storch et al., 2002; Antle and Silver, 2005; Fuller et al., 2006; Sobel et al., 2017; El Cheikh Hussein et al., 2019). SCN is primarily stimulated by the light entrainments around the day/night periods and passing the cues to a dispersed network of peripheral clocks located in distal tissues in the mammalian systems. With the day/night cycles, the SCN-master pacemaker plays as a vital timekeeper to regulate sleep/wake cycles and many crucial physiological activities (Antle and Silver, 2005; Fuller et al., 2006; El Cheikh Hussein et al., 2019). Temporal tuning of the distal tissue-specific peripheral clocks upon robust expressions of circadian genes is indeed tricky to be explicated at the dynamic frame. Implications in several transcriptional regulators have been well characterized in controlling the ticking of peripheral clocks and coordinating tissue-specific temporal activities (Sobel et al., 2017). Moreover, the transcriptional controls have repeatedly emerged as the primary and fundamental phenomenon backing at the circadian output output (Storch et al., 2002). It is essential to invade deeper to expose the reasons supporting the divergent ranges of functional level executions typically governed by the fewer components of the same molecular architecture. It is also important to consider all possible molecular influences such as posttranscriptional, translational, and posttranslational reactions along with the transcriptional process (Menet et al., 2012; Nguyen et al., 2014; Partch et al., 2014).

The same gene was found to be expressed differently in different tissues. The tissue-specific expressions of any target gene displayed the different levels of expressions having significantly different dynamic trends in their expression signatures. The plausible reasons for such discrepancies can be well explained because the underlying regulatory TF-logics were different across different peripheral tissues. Thus, the influence of TF-logics to drive the different tissue-specific gene expressions at different dynamic scale have been established. It has been depicted that the target gene *Cry1* has been expressed among heart, lungs, liver, adrenal gland, kidney, hypothalamus, brain stem, and cerebellum (Figure 4). The *Cry1* was found to be expressed in heart, lungs, liver, adrenal gland, and kidney having similar dynamic trends, as the regulatory TF-logic underlying the *Cry1* regulation has remained same as (CRY1.>.CLOCK) (Figure 4A). However, in the hypothalamus, the dynamic trends of the expression were different, as the regulatory TF-logic was changed to (CRY1.>.CRY2) (Figure 4B). In cerebellum and brain stem, two distinct signature dynamic trends were also noticed wherein the *Cry1* was regulated by two different logics in those two tissues, (CRY1.|.CRY2) and (CRY1.>.PER2), respectively (Figures 4C and 4D). Therefore, the variations of the underlying TF-logics
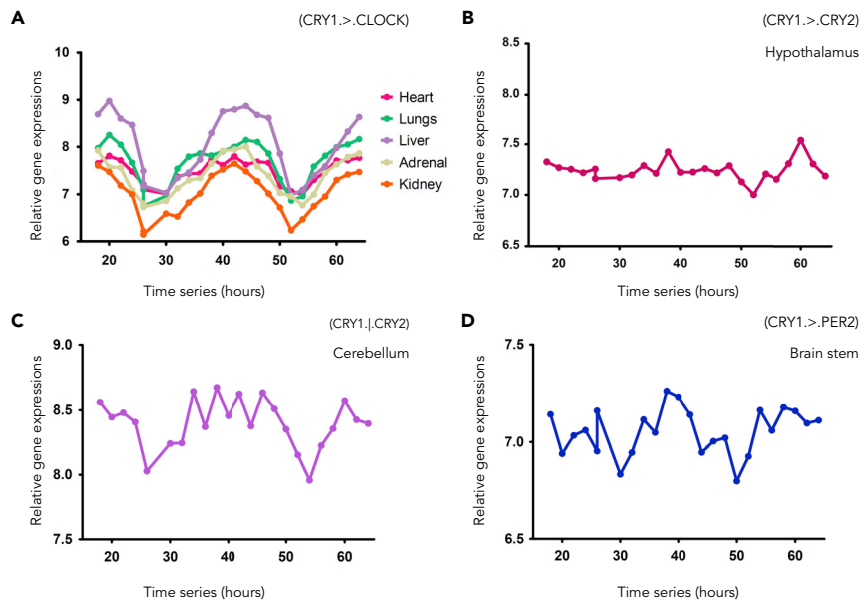
Figure 4. Tissue-specific expression of *Cry1* across different tissues

The target gene *Cry1* was expressed in different tissues displaying different expression dynamics, and the underlying regulatory TF-logics were also varied among different tissues. It has been depicted that the target gene *Cry1* has been expressed among heart, lungs, liver, adrenal gland, kidney, hypothalamus, brain stem, and cerebellum. See also Table S5.

(A) The *Cry1* was found to be expressed in heart, lungs, liver, adrenal gland, and kidney having similar dynamic trends, as the regulatory TF-logic underlying the *Cry1* regulation remained same as (CRY1.>.CLOCK).

(B) In the hypothalamus, the dynamic trends of the expression were different as the regulatory TF-logic was changed to (CRY1.>.CRY2).

(C) In the cerebellum, a distinct signature dynamic trends were also noticed wherein the *Cry1* was regulated by (CRY1.|.CRY2).

(D) In the brain stem, another distinct signature dynamic trends were also noticed wherein the Cry1 was regulated by (CRY1.>.PER2). Therefore, the variations of the underlying TF-logics can be considered as the pivotal driving force to differentially regulate the dynamic gene expressions across different tissues.

can be considered as the pivotal driving force to differentially regulate the dynamic gene expressions across different tissues.

The well-predicted circadian transcriptional regulatory logics are tissue-specific. The distribution of different regulatory logics for the same target gene was found to be different across different tissues. The expressions of the TGs across those 11 different tissues were depicted (Table S5). This indicated the possibility of the same gene to be regulated by different molecular regulatory logics in different tissues (Table S5). The gene expression regulation dynamics of those genes with circadian fluctuations were governed by a different set of regulators in a tissue-specific manner.

DISCUSSION

Perturbed circadian rhythms are becoming increasingly evident as deleterious events in the pathophysiology of sleep disorders. Adjusting those perturbed rhythms to a healthy one can be promising to treat different circadian rhythms misaligned disorders including sleep disorders. Clinically, to achieve this, we must need to have a comprehensive understanding of the molecular controls underpinning the circadian rhythms. It is complicated and systematic involving thousands of genes and their concerted interplays. Despite many experimental affirmations about the circadian transcriptional controls, there still remains an interesting question unexplored that how do these few regulatory components belonging to the same molecular architecture are capable to drive such divergent functions? Their regulatory mechanisms and combinatorial interplay have not gained any comprehensive attention yet. Therefore, in our study, we have deployed the LogicTRN framework to expose the quantitative transcriptional regulatory landscape underpinning the genes having circadian rhythmic fluctuations. We developed a systematic approach

considering core circadian TFs and their relational interplay to decode the pattern of combinatorial transcriptional regulations underpinning circadian rhythms. In future, it may unleash a potential to therapeutically target the molecular regulators to adjust the circadian rhythms to treat sleep disorders.

Reconstruction of qTRNs Is Robust, Physiologically Relevant, Scalable, and Reproducible

Our study using LogicTRN (Yan et al., 2017) helped to characterize those combinatorial patterns of interactions among the TFs efficiently. It has revealed additional molecular information about the transcriptional regulation, namely logic layers. This has unveiled the deep instrumentation of the core pacemaker systems and their temporal dynamic controls at the systems level.

We subsequently extended our computational framework to reconstruct the qTRNs and to predict the dynamic behaviors of those genes expressions. The qTRN essentially indicated the core hubs of the regulatory TF logics and the network of their target genes. It also helped to portray the dominant TF-logics, which may be presumed to exert the pacemaker functions in maintaining circadian fluctuations in the cellular environment. Reconstruction of such qTRNs devises a powerful tool to decode the circadian transcriptional regulations. The introduction of the additional logic layer has emerged as essential information, which was not exposed before. And, it holds a strong capacity to control the gene expressions. Relying on this qTRN, we have identified and characterized the broad range of genes potentially exhibiting circadian rhythmic fluctuations. We identified 461 genes to have circadian fluctuations in their expressions. It offered insights about the regulation and orchestration of core molecular clocks and peripheral clocks that incorporate many combinatorial strategies to regulate their transcriptional outputs. The modes of transcriptional regulations of different genes with circadian fluctuations were tightly controlled by the influence of TF logic or their combinatorial logics. The major driver TF logics were identified for the well-characterized genes. These drivers may signify the deep instrumentation of the core pacemaker of the circadian rhythmic controls. Therefore, the fine mechanistic insights about the transcriptional regulators and their interplay have become very important to be devised.

The LogicTRN algorithm (Yan et al., 2017) was extensively employed in our study and we have improved the existing framework of LogicTRN to comprehensively reconstruct the qTRNs and to predict the accurate dynamic profiles for gene expressions. It precisely quantifies the TF-DNA binding occupancy and caters a quantitative understanding of the combinatorial regulatory landscape for gene regulation. Thus, this foundation has been leveraged to innovate a strategy to estimate the influence of any TF or combination of TFs to their network of target genes at a dynamic scale. It has been introduced in our study as a virtual knockout approach. In the view of the methodological benefits, our computational approach is robust, scalable, and biologically relevant. It can be diversely employed in high-throughput time-series gene regulation data analysis to investigate the potential molecular targets. It can be applied in diverse fields of applications before proceeding to the experimental trials and errors. Thus, it promises to save valuable time and resources.

An Advanced Direction for Engineering at the Transcriptional Controls

Our results have suggested that the same TFs does not always mean to control the same group of TGs. Rather, the fashions of their interactions are the determinants to govern these regulatory behaviors. Such a fine dynamic resolution was not considered earlier. This certainly supports another formerly unheralded association between the variations in gene expressions and circadian oscillation generations. This study showed that different pattern of stochastic interactions among the TFs contributes substantially to the dynamic variations in circadian periodicity. Our study with vKO mutants has also established a link that controlling transcriptional regulators may directly influence circadian periods. We have represented an integrative approach of transcriptional simulations with virtually knocked out circadian TF mutants. The comparative impact of different knockout species and their wild type for a specific gene was carefully evaluated. A scoring method was also developed to quantify this influence and then they were ordered. Our results helped to identify the best transcription factor combination that can effectively modulate the circadian rhythms. Of them, the CLOCK/CRY1 vdKO preserves the highest capacity to modulate the circadian rhythm dynamics. This has offered a robust screening of the genes, especially the essential genes to investigate their functional influence quantitatively on the network of the target genes at a dynamic scale. Our results demonstrated the efficiency to estimate the influence of different virtually knocked out mutants on individual circadian gene expression profiles having circadian fluctuations. Our results helped us to assess the specific TF regulators and the magnitude of their contribution in

the transcriptional regulations of the genes with circadian fluctuations. This may potentially enable us to develop further decision to target the rightmost or at least several combinations out of the top-ranked TF combination to be targeted for further experimental validations. And this strategy could be plausibly exploited to attain the desired phase shifts for any specific circadian fluctuation of any gene of interests linked to any diseases. Broadly, this may facilitate us to adjust the misaligned circadian rhythm to a correctly aligned one with precise directionality. Our results are quite consistent with the experimental evidence. For instance, our analysis validated the *Apoc3* function as one of the key players to control cholesterol and bile acid metabolism rhythmically, which was a well-established fact with several experiments (Cho et al., 2012). Also, *Lpin1* was validated to exhibit circadian fluctuations in its expressions in liver and adipose (Zhang et al., 2008; Champier et al., 2012). It was also well predicted across those three different conditions. Interestingly, the prediction of their regulatory TF logics across the wild type, virtually knockout mutant, and experimentally knockout mutants also shared the consistency. So, employing this vKO strategy may help us to know more about the specific TF regulators and the magnitude of their contribution in the transcriptional regulations of the genes with circadian fluctuations. Identifying the best TF-combination can be achieved by the experimental method certainly. However, that would be extremely tedious as well as time and resource consuming. And, the success rate is not well guaranteed keeping pace with the resources utilized. Therefore, as an alternative way, we have developed a quite physiologically relevant algorithm that relies on quantitative transcriptional regulatory networks to screen the best regulatory TF-combination having the best efficacy to modulate the circadian rhythmic dynamics.

This work was instituted to establish the connection of the different clock-controlled genes expressed across the distal tissues. With our pilot studies, we tried to enlighten on the direction of the tissue-specific tuning of the peripheral clocks. Indeed, it was quite challenging to consolidate the molecular information to reproduce a meaningful direction. However, with our analysis, we have exposed some interesting facts about the interplay of the different transcriptional regulators and their target genes' expression at dynamic scale across 11 tissues. Our results indicated that the same target gene may get expressed differently having a different dynamic trend in their expressions across tissues. And, this phenomenon can be plausibly explained by the TF-logics. In our analysis, it was found that when a target gene was expressed under the same TF-logic, it tends to possess a similar dynamic trend of expressions in tissues. However, when that same target gene was expressed in different tissue with different TF-logic, their dynamic trend is changed. Therefore, the underlying TF-logics may be perceived as the pivotal driving force that differentially regulate the dynamic gene expressions across different tissues. Also, it has been seen in our results that the same target gene is regulated by different sets of TF-logics across different tissues. And, on the other way around, across different tissues, it has been found to have different groups of target genes under different regulatory TF-logics. Thus, the influence of TF-logics to control the gene expression can be presumed as tissue-specific.

These foundations direct an avenue for researchers to engineer the molecular controls of the circadian clockwork further to modulate its outputs as per clinical needs, such as reversing the altered period lengths in sleep disorders especially in the anticipated directions for modulating periodicity could also be achieved. Besides, it may facilitate in screening the right-fit druggable TFs that can effectively modulate the circadian periods to realign the rhythmic discrepancies. Furthermore, it may unleash a potential to therapeutically target the molecular regulators to adjust the circadian rhythms to treat the different spectrum of disorders related to misalignment of circadian rhythms.

Limitations of the Study

In this study, we have furnished a physiologically relevant algorithm and proposed an integrative quantitative framework to investigate the deep mechanistic insights about combinatorial transcriptional regulations for circadian gene expressions. This has led a comprehensive perspective from molecules to systems. Although the core algorithms used in this study potentially overcome four prominent challenges associated with existing approaches considering the transcriptional processes are highly dynamic in the cellular environment, regulatory effects of any TFs on their target genes are essentially nonlinear, transcriptional regulators often work cooperatively on their target genes, and the kinetic parameters are often unknown, which demands to be estimated. Despite such strong considerations, the proposed foundation shares some limitations. The accuracy of prediction is directly proportional to the number of time points. The precision of LogicTRN model analyze the genome-scale time-series data for biological rhythms, and the

datasets are expected to preserve some features as per the standard guidelines (Hughes et al., 2017). The preferred features for precise predictions with LogicTRN model on time series data include the following: (1) high-resolution temporal datasets having more time points offer better purposes while analyzing the dynamic expression behaviors of rhythmic transcripts; (2) lower the sampling frequency, better the precision in capturing the dynamic events; (3) larger intervals in sampling (usually > 2h interval may miss several crucial transcriptional events in those genes' rhythmic expressions), which may deviate from the physiological relevance; (4) statistically, the greater the number of data points, the more accurate the predictions as well. Therefore, deploying our LogicTRN-based method must return better resolution with the datasets sharing maximum numbers of time points for gene expressions. Particularly, in this study, we have used static TF-DNA data, wherein the physiologically relevance and robustness are expected to be maximized upon using dynamic ChIP-Seq datasets. And, from our study results, complex logics may not be entirely consistent across all the different datasets used in our validations. These variations could be addressed with two plausible explanations: (1) limitations of our methods comprising with the only TFs amid the transcriptional regulations, wherein it is quite possible that other upstream regulatory influencers exerts significant influences to those TFs including but not only limited to protein-protein interactions, histone modifications those were not included in our current framework used in this study; and (2) there could also be certain degrees of inconsistencies among the experimental conditions for each of the datasets that was merely not identified within our strengths. Certainly, it will be interesting to explore and extend our foundation to accommodate more molecular information to reconstruct a more precise and relevant framework to predict transcriptional regulatory landscapes.

Resource Availability

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Dr Hailong Zhu, hlzhu@hkbu.edu.hk.

Materials Availability

This study did not generate any new unique reagents and materials.

Data and Code Availability

The LogicTRN codes are available at https://github.com/Solipugids/logictrn_demos.git. Any further assistance can be directly made to Hailong Zhu. The figures displayed in this research paper is also available in Mendeley Data DOI, https://data.mendeley.com/datasets/hs66b42sf7/2. Other transcriptomic and ChIP-Seq data used in this study were published previously and are publicly available on GEO (time-series gene expression data: GSE11923, GSE54650, GSE13062 and TF-DNA binding ChIP-Seq data: GSE39860). Other supporting data are available in the Supplemental Information. Further details can be made available from the authors upon genuine request.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101490.

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AUTHOR CONTRIBUTIONS

D.C. envisioned the concept. D.C. and H.Z. refined the idea and designed the study. D.C. wrote the manuscript. C.W. and D.C. edited the manuscript. All authors discussed data, contributed to editing the manuscript. H.Z. developed the core algorithms. D.C. and C.W. performed all data collection, processing, and analyses. H.Z., A.L., and C.W. supervised the work. H.Z., and A.L. directed the team, handled administrations, and provided guidance and funding.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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Supplemental Information

Identifying Transcription Factor Combinations

to Modulate Circadian Rhythms by Leveraging

Virtual Knockouts on Transcription Networks

Debajyoti Chowdhury, Chao Wang, Aiping Lu, and Hailong Zhu
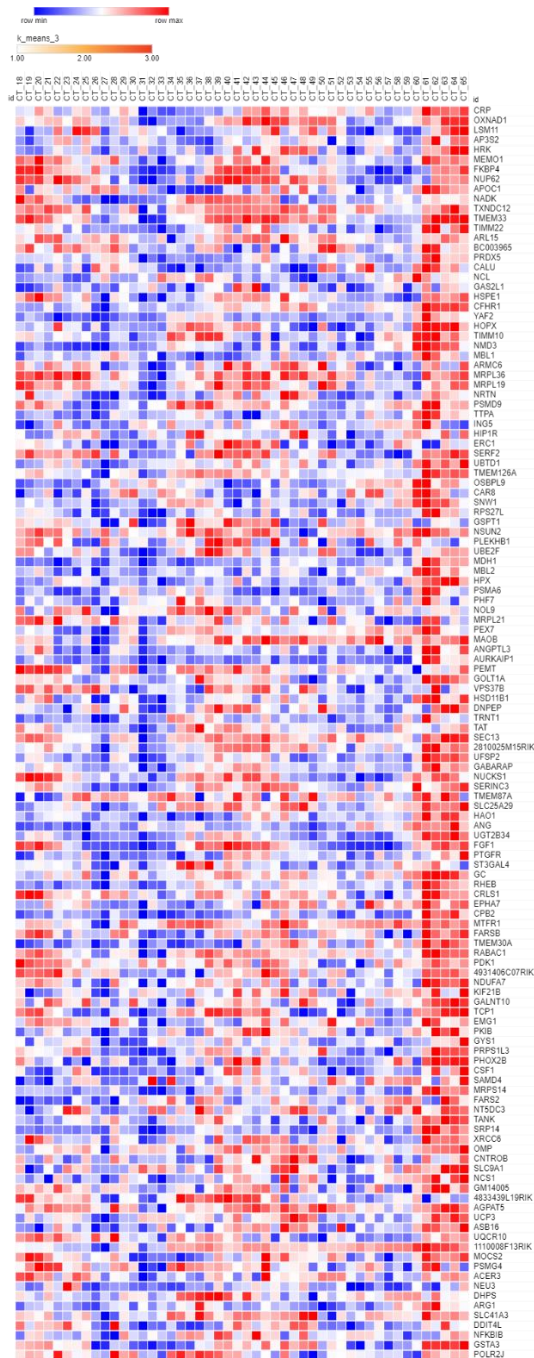
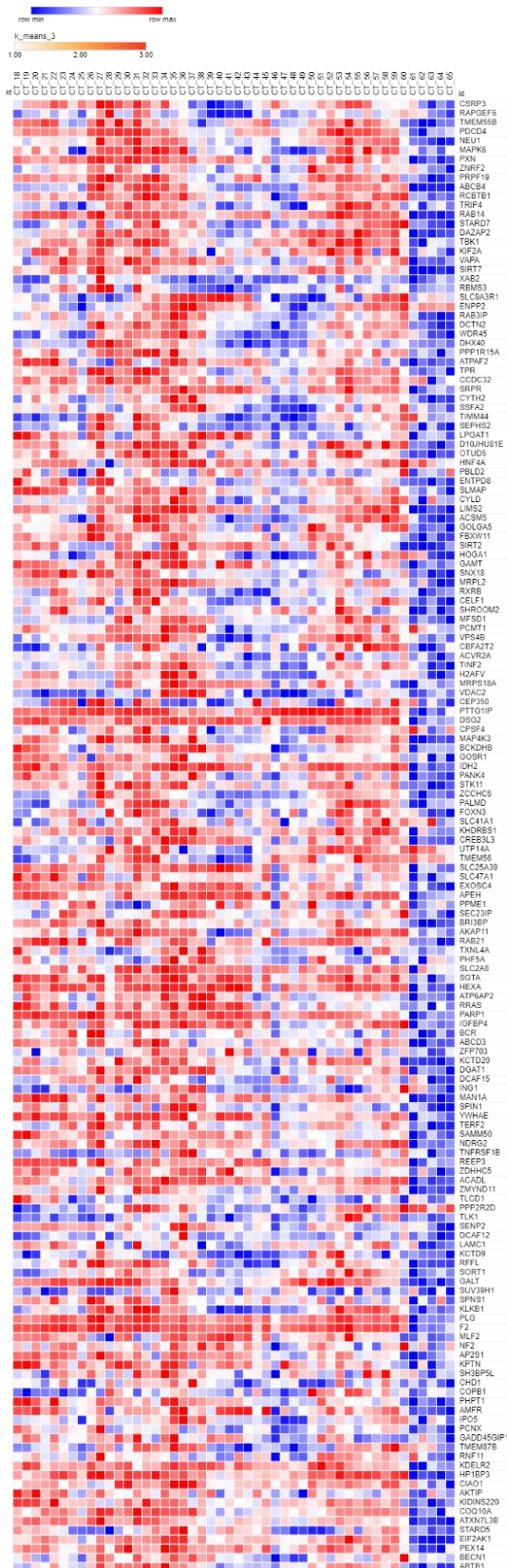**Figure S1. Clustering of the well-predicted genes having circadian rhythms (cluster 1). Related to Figure 1C.**

The genes belonging to the cluster 1 (n=160) out of the well-characterized 461 genes with having circadian fluctuations. The group of 160 genes belonging in the cluster one has been linked with having maximum enrichment for cholestasis, a decreased biliary flow, cardiomyopathic syndromes and several types of cancers including, squamous cell carcinoma, adenocarcinoma, melanoma, and pancreatic cancer with having p-value < 0.05. They showed likelihood in developing autoimmune disorders, episodic ataxia, and central hypoventilation syndrome. Out of which, biliary dysfunctions and associated metabolic diseases were strongly supported with having a link to the genes under tightly controlled by CRY1, and several other circadian TFs as well. Cardiomyopathy syndromes have also been largely connected as a result of circadian disruptions. Also, the development and progression of adenocarcinoma, melanoma have been supported to be linked with disruption of circadian rhythms. The KEGG pathway analysis indicated the most enriched pathways related to those 160 clustered genes are AMPK signalling, Cholesterol and fatty acid metabolism, Necroptosis pathways among the most relevant one at p-value < 0.005.
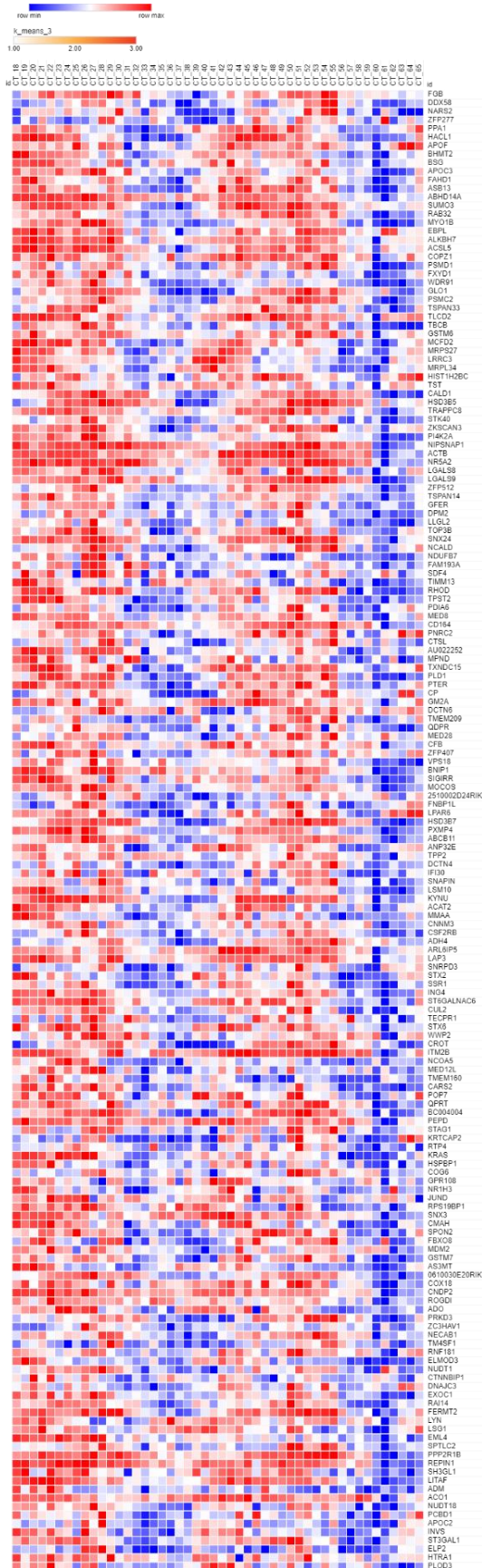
The genes belonging to the cluster 2 (*n*=173) out of the well-characterized 461 genes with having circadian fluctuations. The group of 173 genes belonging to the cluster two showed maximum enrichment for several neurodegenerations (most likely to develop macular degeneration, ataxia), thrombophilia among the coagulative cardiovascular diseases and leukaemia with having *p*-value < 0.5. Few genes from this cluster have been well characterized to exert similar disease profiles with experimental supports. For example, *Spon2* gene has been established to exert significant roles in developing cardiovascular diseases. The clinical significance of the gene, *Spon2* has also been well characterized by our study as possessing the tight circadian rhythmic fluctuations in its expressions and also possess circadian outputs. The KEGG analysis supports the OMIM disease enrichment based on this cluster by indicating the most likely terms associated with this cluster includes, the SNARE interactions in vesicular supports, cholesterol, sphingolipids, and fatty acid metabolisms at *p*-value < 0.005.

Figure S3. Clustering of the well-predicted genes having circadian rhythms (cluster 3). Related to Figure 1C.

The genes belonging to the cluster 3 (*n*=128) out of the well-characterized 461 genes with having circadian fluctuations. This group of genes belonging to the cluster three exhibited maximum enrichment for diabetes, myocardial infarction, obesity, and glycogen storage diseases at *p*-value < 0.09. And, few genes, *Gys1, Ucp3, Mbl2* from this cluster were most relevant with developing those diseases. They are linked to the metabolism of xenobiotics, coagulation cascades, and ribosome transports at *p*-value < 0.005 most likely.
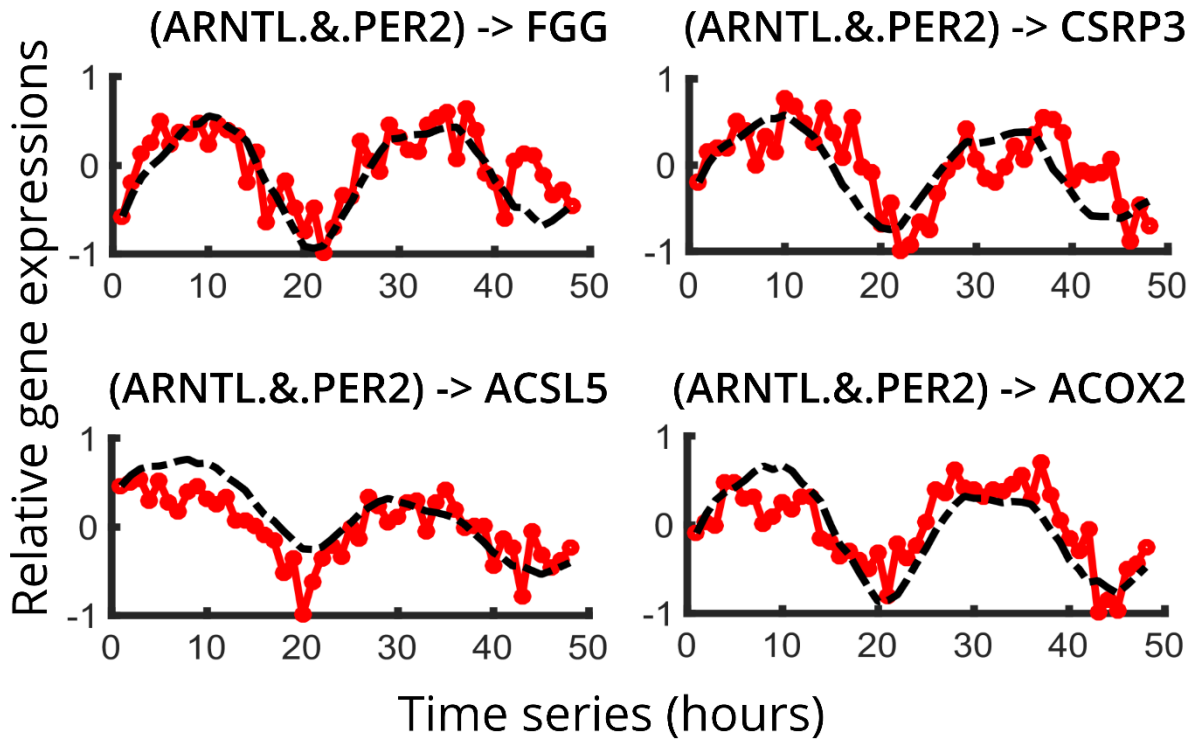
Figure S4. The qTRN predicted dynamic gene expression profiles for the well-characterized genes with circadian fluctuations. Related to Figure 2A.

The genes under the control of (ARNTL.&.PER2) regulatory logic have been shown here. The target genes, *Clock, Fgb*, *Fgg, Csrp3*, *Acsl5* and *Acox2* were transcriptionally controlled by the same regulatory logic, (ARNTL.&.PER2). These target genes under the same regulatory logic also shared very similar and well predicted dynamic features. Thus, the binding to those 10 TFs are most likely to participate in various circadian rhythms related functions. Of them, the *Clock* gene was experimentally supported to be regulated by the influence of PER1 and ARNTL. The *Acsl5* was also experimentally supported to be strongly regulated by the core clock-controlled regulators including PER1 and strongly endorsed to have vital circadian output functions in hepatic fatty acid metabolisms, temporal controls on hepatic catabolic and anabolic pathways and lipid metabolisms.
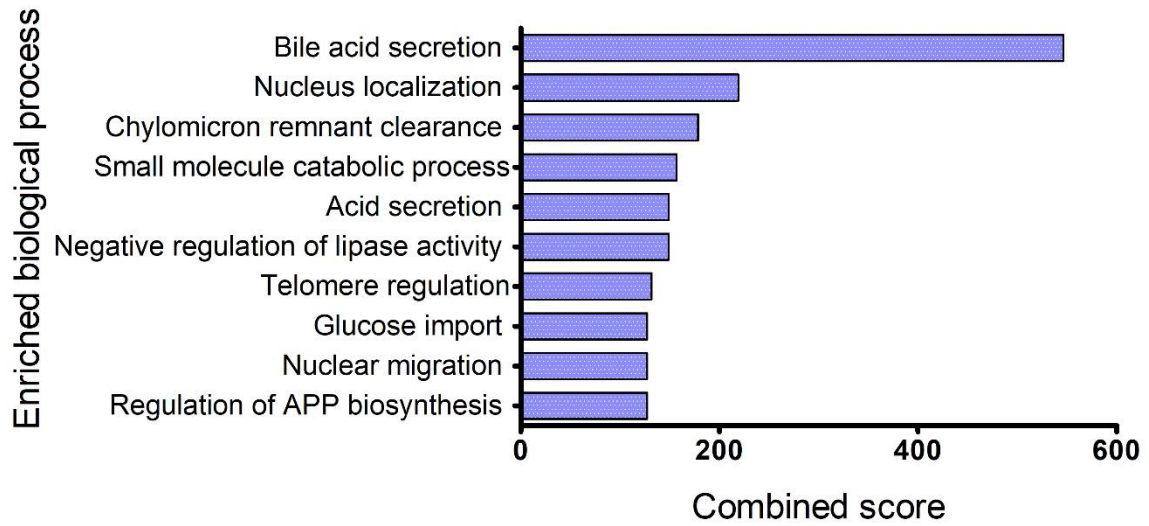
Figure S5. CRY1-associated functional enrichment on the qTRNs. Related to Figure 1C, and Figure 3C.

The most enriched biological processes related to the sets of the gene under the regulatory controls of the CRY1. It indicates the bile acid secretion to be the most relevant physiological process to be influenced.

TRANSPARENT METHODS

Studying the circadian biology is strictly time driven. Thus, having a dataset with a maximum number of time points must produce a better resolution of the analysis. It will also help in predicting the dynamic profiles more accurately. Here, our methodology integrated the high-throughput time-course gene expression data from microarray and high-throughput TFs-DNA binding signals from ChIP-Seq experiments to quantify TFs-DNA binding occupancy. And, as an output, it is returned with the all-inclusive combinations of regulatory TFs-logics that drive the genes' transcriptional regulations. Using these combinations of TF-logics, the qTRNs were reconstructed and the dynamic expression behaviours were well-predicted for those TGs within the qTRN. Then, vKO mutants-constituted transcriptional simulations were performed to quantitatively estimate the influence of different sets of TFs on perturbing the rhythmic dynamics of the genes with circadian fluctuations. The datasets, methods related to computational modelling, analysis and evaluation protocol are described as below.

Datasets, data processing and annotations

Datasets

Two publicly available datasets were used in this study. The GSE11923 for time-series gene expression data from microarray experiments(Hughes et al. 2009). The GSE11923 possessed 48-time points and the optimal resolution of 1-hour interval between each sampling. Thus, with this dataset, we were able to get a 48-hours long dynamic snapshot of the mouse gene expressions which is extremely contextual in terms of circadian study. And, the high-throughput TF-DNA binding ChIP-Seq experiments with the mouse to understand transcriptional architecture and chromatin landscape of the core mammalian circadian clock(Koike et al. 2012) were used. The time-course gene expression data were sampled every hour for 48 hours from mouse liver with having a minimum of three biological replicates at each time point(Hughes et al. 2009). And, the TF-DNA binding data(Koike et al. 2012) were obtained for ten circadian TFs, namely, CLOCK, ARNTL, CRY1, CRY2, PER1, PER2, NPAS2, CREBBP, EP300, POLR2A to be integrated as an input into our LogicTRN(Yan et al. 2017) algorithm.

Processing of the time-course gene expression data

The Gene expression omnibus (GEO) accession number for the mouse liver microarray was presented here, GSE11923 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE11923. This time-course gene expression dataset contained 48 samples, which were originally obtained using the platform, Affymetrix Mouse Genome 430 2.0 Array chip (Platform ID: GPL1261-[Mouse430_2]) sharing 22626 probe sets including several unmapped ones. Samples were collected every hour for 48 hours from 3-5 mice per time point from the liver. Samples were pooled and analyzed using Affymetrix arrays(Hughes et al. 2009). High-temporal resolution profiling was performed on mouse liver to detect rhythmic transcripts. The raw data from the dataset, GSE11923 were separately processed using the Robust Multi-Array Average (RMA) probe summarization algorithm to obtain normalized expression values.

Processing of the TF-DNA binding signals from ChIP-Seq data

Published datasets of ChIP-Seq(Koike et al. 2012) of the 10 core circadian TFs, CLOCK, BMAL1, CRY1, CRY2, PER1, PER2, NPAS2, EP300, CREBBP, POLR2A in the mouse liver were obtained in this study. The ChIP-Seq peaks were identified from uniquely mapped reads without duplicates using MACS(Koike et al. 2012).

LogicTRN, TF-logics, dynamic predictions, and evaluation

Our recently developed methodology integrates the high-throughput time-course gene expression from microarray and/or RNA-Seq experiments, and ribosome-footprinting data(Iyer and Grummt 2017; Sobel et al. 2017; Jang et al. 2015; Janich et al. 2015) to replace specific TF-genes expression information, high-throughput TFs-DNA binding signals from ChIP-Seq experiments, and upstream protein-protein interactions including TFs-TFs interactions, and interactions among TFs with other proteins before binding to their TGs as well as their associated influences to modulate TFs' DNA binding ability together to quantify TFs-DNA binding occupancy(Di lena, Nagata, and Baldi 2012). And, as an output, it was returned with the all-inclusive combinations of regulatory TFs-logics that drive and/or regulate the circadian gene expression. From, these combinations of logics, the qTRNs were reconstructed and the detailed mechanistic insights of the gene transcription process were interpreted. Moreover, the quantitative framework also offered the precise prediction of the dynamic profiles of circadian genes expression.

With this way, we stepped forward towards a more comprehensive understanding of such transcriptional variability and its dynamic behaviours in circadian rhythms generation. Our novel integrative approach offers much better insight into the regulation and orchestration of core molecular clocks and peripheral clocks that incorporate many strategies to regulate their transcriptional outputs. Therefore, decoding them smartly may contribute to the flexible necessity to infer tissue-specific spatio-temporal regulation of circadian physiology and associated behaviours. Besides, we can also comprehensively identify the components constructing the central-circadian-pacemaker and elucidate their interplay and core mechanism of them with the extensive application of our theoretical framework.

In transcription regulatory network, the rate of newly synthesized mRNA is equal to the rate of transcription initiation rate if all pre-mRNA molecules are processed to mature mRNA(Yan et al. 2017). Let $y_m(t)$ represent the gene expression at time t, the transcriptional kinetics can be expressed as an ordinary differential equation (ODE)(Ben-Tabou de-Leon and Davidson 2009a),

$$dy_m(t)/dt = I_s(t) - k_{dm}y_m(t),$$

where $I_s(t)$ is transcript initiation rate, and k_{dm} denotes mRNA degradation rate. Generally, the transcription initiation rate is mainly controlled by TF binding, which is consisted of binding occupancy, $Y_m(t)$ at time t and regulatory strength, K_b. Here, the transcription initiation rate $I_s(t)$ can be represented as the regulatory function below(Yan et al. 2017; Ben-Tabou de-Leon and Davidson 2009b):

$$I_s(t) = I_{max}(1 - exp\left(-\frac{k_b Y(t - T_m)}{I_{max}}\right))$$

where I_{max} is the physical limit of transcript initiation rate determined by the RNA elongation speed and the size of the polymerase, k_b is the TF activation strength, and T_m the transcriptional delay.

When two TFs are engaged in regulating a gene, the TFs might interact with each other in different ways. TF interactions are often represented as basic logics including AND, OR, and NOT(Yuh, Bolouri, and Davidson 1998, 2001; Buchler, Gerland, and Hwa 2003; Istrail and Davidson 2005). The AND logic describes the situation that the gene is only activated when the two TFs concurrently binding to the gene promoter, and OR logic represents that the gene can be independently activated by either of the two TFs, while NOT logic characterizes the inhibitive operation. By applying the Taylor expansions and a series of mathematical transformations, we have obtained a model equation of gene transcriptional regulation(Yan et al. 2017):

$$\hat{y}_m(t_l) = \sum_{j=1}^{N_Z}\beta_j \cdot Z_j + (1 - k_{dm}) \cdot y_m(t_{l-1}) + \varepsilon$$

Where, $l = 2, \cdots, L$ are the indices of time points, $\hat{y}_m(t_l)$ is the predicted expression value of the target gene at a time t_{l-1}, $y_m(t_{l-1})$ is the actual expression level at a time t_{l-1}, k_{dm} is the gene degradation rate, β_j is the model coefficient, which is also the function of transcriptional kinetic parameters. Z_j is the function of TF-DNA occupancies, N_Z is the number of all the regulatory logics that can be potentially involved in regulating the target gene.

Usually, genes are controlled by multiple TFs in a combinatorial way. The functions of combinatorial regulation are commonly represented as AND OR NOT basic logic functions. The AND logic describes the situation that the gene is only activated when the two TFs concurrently binding to the gene promoter, and OR logic represents that the gene can be independently activated by either of the two TFs, while NOT logic characterizes the inhibitive operation. These three logic functions can be described as bellow:

AND (denoted by A & B):

$$I_s = I_{max} \times \left(1 - exp\left(-\frac{k_b^{A\&B}}{I_{max}}Y^A(t - T_m) \times Y^B(t - T_m)\right)\right)$$

OR (denoted by A | B):

$$I_s = \frac{I_{max}}{2} \times \left\{ \left(1 - exp\left(-\frac{k_b^A}{I_{max}} Y^A(t - T_m) \right) \right) + \left(1 - \left(1 - exp\left(-\frac{k_b^B}{I_{max}} Y^B(t - T_m) \right) \right) \right) \right\}$$

NOT (denoted by A > B):

$$I_s = I_{max} \times \left(1 - exp\left(-\frac{k_b^A}{I_{max}} Y^A(t - T_m) \times \left(1 - Y^B(t - T_m) \right) \right) \right)$$

When a gene regulated by two TFs, it can form at most six unique regulatory logics (URLs) according to the definitions of transcription regulatory logic functions discussed before. Each URL have a probability of occupancy from 0 to 1 in gene regulation.

Interpretation of the TF-logic symbols used in this study: Three logical operators were used in our study to explain the combinatorial interaction fashion among the TFs. These three logic operators include "AND" (&), "NOT" (>), and "OR" (|). They are explained with a context of gene regulation having circadian fluctuations with context. First, the *Fahd1* gene was regulated by the TFs logic, "CRY1.&.PER2". The expression of the *Fahd1* gene was controlled by two candidate TFs, CRY1 and PER2. These two TFs formed "AND" logic while regulating *Fahd1* gene. Thus, only when these two TFs, CRY1 and PER2 was bound together (collaboratively) to its target gene, *Fahd1*, they activated the expression of *Fahd1*. Second, the *Sephs2* gene was regulated by the TFs-logic, (CRY2.>.PER2) connected through "NOT" logic. In this condition, *Sephs2* was expressed only when CRY2 was bound to its promoter but the PER2 did not bind. However, the *vice-versa* condition was not likely to happen when two or multiple TFs are connected through "NOT" logic. Third, the expression of *Dcaf12* gene corresponds to the "CRY2.|.CREBBP" logic. The two TFs, CRY2 and CREBBP were concerted through "OR" logic. In this case, *Dcaf12* gene was expressed in two probable conditions, either the CRY2 or CREBBP; and/or both CRY2 and CREBBP were bound together.

Quantitative network modelling and reconstruction of qTRNs

The method used for model construction and prediction have been discussed in detail previously(Yan et al. 2017). Here, we described the extended implementation of that method to study the dynamic prediction of gene transcription regulation mechanism at a dynamic scale and to reconstruct the transcriptional regulatory networks relying on quantitative parameters. At first, all the 10 core circadian TFs (CLOCK, BMAL1, CRY1, CRY2, PER1, PER2, NPAS2, EP300, CREBBP, POLR2A) were used as candidate regulators for the rest of gene. By using the time-course gene expression and TF-DNA binding signals, all the dynamic TFs-occupancies were computed. Then a series of model equations of the pairwise-logic model was formed. By constructing the model equation, the coefficient matrix was obtained by conducting LASSO regression on the group of model equations of any target gene(Yan et al. 2017). The confidence value of each unique regulatory logics was calculated according to the coefficient matrix. Finally, both the logic functions with regulator TFs were determined for each TGs to reconstruct the regulatory network. And, they were visualized using Cytoscape v3.5.1.

In our study, we analyzed the time-series gene expression data and TF-DNA binding signals from ChIP-Seq. The time-series gene expression data were analyzed using R programming (R v3.4.3 and R studio v1.1.414) and ChIP-Seq data were analyzed using MACS programming in R. Then, we integrated them into our algorithm to calculate the TF-DNA binding occupancy at each time point. The simulations were performed using a MATLAB (R2016a). It is a quantitative estimation over the time course. Based on this, we predicted the dynamic gene expression behaviours. We have analyzed all the predicted dynamic profiles for the gene expressions along with their original signals from microarray experiments. This is one of the default outputs of our algorithm to represent the predicted dynamics along with their original expression profiles. Based on this judgment, we have carefully curated the very well-predicted dynamic profiles wherein the dynamic predictions are well-matching with the original gene expression profiles having cyclical expression patterns over the 24-hours. As our predictions are based on 10 circadian TFs, we have claimed that those well-predicted genes are likely to play roles in circadian rhythms and have circadian or daily fluctuations.

Construction of virtual knockout mutants for core circadian TFs and transcriptional simulations

Based on the qTRNs of the genes with circadian fluctuations, we developed the approach of vKO to evaluate the influence of a TF or TF-combinations in regulating the gene expressions of the genes

having circadian fluctuations at dynamic scales. In vKO mutant construction, firstly, we used LogicTRN method to predict the TF-logics responsible for driving the gene expressions regulations(Yan et al. 2017). And, this data was successfully reflected with the wild-type (WT) conditions. we have considered minimum one to a maximum combination of three TFs knockout conditions. We named them as virtually single TF knockout (vsKO), virtually double TFs knockouts (vdKO) and virtually triple TFs knockouts (vtKO).

For vsKO mutants, it was a more straightforward way to construct the virtual mutant. Firstly, we took out a TF from the qTRN and predicted the dynamic profiles of the genes with circadian fluctuations using the perturbed qTRN using MATLAB (R2016a). Knocking out a TF is nothing but to make their corresponding TF-gene expression values to zero. So that, in the further transcriptional simulation, that TF does not exhibit any sort of influence in the qTRN. This was considered as a perturbed one. Thus, by comparing the phase shifts from the dynamic profiles between the original rhythms out of WT conditions and the perturbed one from the mutant conditions, a score was calculated. It represented the extent of influence of the different TFs knockouts. Thus, the top-ranked TFs and/or TF-combination were identified using the scores in terms of modulating circadian rhythms. Similarly, for vdKO mutant construction, we figured out all possible combinations of two TFs based on the baseline WT logic prediction data. Accordingly, those combinations of the double TFs were virtually knocked out by replacing their corresponding TF-gene expression values to zero. And, all the combinations were subjected for the transcriptional simulation using LogicTRN based framework(Yan et al. 2017). And, again, for the vtKO mutant constructions, all possible three combinations were knocked out sequentially. And, each condition with individual TF or TF combinations were subjected for transcriptional simulation separately. Also, they were individually subjected for prediction of the dynamic profiles of the genes with circadian fluctuations using the perturbed qTRN. And, finally, all the scores were estimated, and they were ranked in a descending way.

Developing a scoring method to evaluate the influence in modulating circadian rhythmicity

After predicting the dynamic expression profiles for those genes, the predicted values were extracted using the LogicTRN algorithm in MATLAB and subjected to MetaCycle program in R (https://cran.r-project.org/web/packages/MetaCycle/index.html) to estimate the lag, period length and amplitude(Wu et al. 2016; Hughes, Hogenesch, and Kornacker 2011). The score was estimated upon comparing the quantification of the phase shifts through the lag values obtained from the predicted dynamic profiles between the rhythms from WT conditions and the rhythms from the mutated or perturbed conditions. The lag values were estimated for all individual genes and all vKO types. Then, for each gene, the difference was computed between the lag value of the vKO types and the corresponding WT conditions. And, the value was divided by the 24 as the typical circadian phase is 24-hours as best considered. Therefore, the concerned individual value reflects the changes in the lag period between the WT type genes and vKO types on a 24-hours scale. And, finally, those values were summed up for all those 461 genes to estimate the cumulative influence on the entire qTRN, that resonates the influence on the overall transcriptional regulatory network level.

Score estimation based on lag values S_{lag} from the analyzed vKO types:

$$S_{lag} = \sum \frac{|LAG_{vKO} - LAG_{WT}|}{24}$$

If the expression of any target gene is silenced, $|LAG_{vKO} - LAG_{WT}|$ should be equal to 24.

Clustering of genes having rhythmic fluctuations and functional enrichment

All the 461 genes those were well characterized to have rhythmic fluctuations and most likely to exert influence in various circadian outputs were subjected for clustering. They were clustered using Morpheus (https://software.broadinstitute.org/morpheus). The clustering method used here was the *k*-means clustering at *k* = 3 and the simulations condition were set to be iterated 1000 times. Thus, those 461 genes were clustered into three groups. These genes were further functionally characterized cluster wise using Enrichr program to find the maximum enrichment for OMIM disease information and to have the most likely pathways using KEGG pathway analysis with using Enrichr(Kuleshov et al. 2016; Chen et al. 2013).

Under the influence of a specific and/or combination of TF-knockout, the group of TGs were subjected to the gene set enrichment analysis (GSEA). Firstly, we considered all ten circadian TFs as per their descending scores in case of single TF knockout mutants. Secondly, for the two TFs knockout mutants,

we considered all the possible combinations of two TFs double knockouts as per their descending scores. Thirdly, for the three TF knockouts, we considered selected combinations of three TFs triple knockout mutants as per their relevance to the circadian rhythmic functions and the scores in descending orders (Table S2). We used Enrich to perform the functional enrichment analysis(Kuleshov et al. 2016; Chen et al. 2013). The most enriched biological processes were included with their combined scores. And, the most enriched phenotypes associated with those set of genes were enlisted with their combined scores. Enrichr method implements three approaches to compute the gene set enrichment (GSE) to precisely assess the significance of the overlap between the input gene list and the gene sets in each gene-set library to rank the term's relevance to the input list(Kuleshov et al. 2016; Chen et al. 2013). Here, we used the terms for biological processes and most enriched phenotypes to be influenced by a descending combined score. A combined score multiplies the log of the p-value computed with the Fisher exact test by the z-score computed by our correction to the test. $c = \log(p) \cdot z$ Where c is the combined score, p is the p-value computed using the Fisher exact test, and z is the z-score computed by assessing the deviation from the expected rank.

Prediction of tissue-specific TF-logics

We have considered the dataset, GSE54650(Zhang et al. 2014) for our tissue-specific study. As per this dataset, a series of circadian gene expressions were documented from 288 samples, covering 12 different tissues. Each tissue was sampled with 2 hours interval for 48 hours' time spans(Zhang et al. 2014). Therefore, 24 samples were considered per tissue from the mouse. And, here in our analysis, we have considered 11 tissues, SCN, hypothalamus, cerebellum, brain stem, heart, aorta, lungs, liver, kidney, adrenal gland, and muscle. We did not consider the fat or adipose tissue in our analysis. The time-series gene expressions from those 11 tissues were analyzed using the protocol as described earlier. The results indicated the predictions of the transcriptional regulatory logic and reconstruction of qTRNs.

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