Understanding Clock Gene Regulation by Constructing a Qualitative Model

Marco Kragten[1], **Nihal Fawzi**[1] and **Bert Bredeweg**[1,2]
[1] Faculty of Education, Amsterdam University of Applied Sciences, Amsterdam, The Netherlands
[2] Informatics Institute, Faculty of Science, University of Amsterdam, Amsterdam, The Netherlands
{m.kragten, n.fawzi, b.bredeweg}@hva.nl

Abstract

We developed a lesson where students construct a qualitative representation to learn how clock genes are regulated. The lesson, designed for upper secondary and higher education, is implemented in the DynaLearn software (level 4), which allows for modelling feedback loops. Students construct the representation step-by-step, guided by a structured workbook and built-in support functions within the software. At each step, the students run simulations to examine system behaviour and reflect on the results through workbook questions. To ensure scientific accuracy, the representation and workbook were evaluated by domain experts.

1 Introduction

Learning by modelling is a valuable approach to learn about dynamic systems. Working with qualitative models has the advantage that students work with vocabulary and inference procedures that closely match human-like thinking [1]. However, modelling system behaviour, even in qualitative terms, can quickly become complex, and students require support to use these models effectively as learning tools [2]. To meet this challenge, we use reference models that serve as a standard on the basis of which the necessary support can be automatically generated [3,4]. Over the past years, various models have been developed, particularly for use in secondary education [5-9].

To serve as a reference model, such models must meet specific requirements [10], including:

- **Graceful progression.** The subject matter must be decomposed into learnable units, yet sufficiently complex, and organized in a sequence addressing the entire phenomenon.
- **Self-contained and manageable**. Qualitative models can generate large state-graphs, or no states at all. To be suitable for learning, models must generate simulations with correct solutions and manageable state-graph sizes.
- **Meaningful.** The decomposition cannot be arbitrary. Each unit must address at least one meaningful aspect of the subject matter.
- **Engaging and curiosity driven.** Surprises can increase students' motivation. Ideally, the units are orchestrated such that they regularly produce intriguing results, which then form the challenge for the next modelling step.

In this paper, we present an advanced reference model that explains the working of the clock genes that regulate the circadian clock [11]. The content of this paper is therefore as follows. Section 2 summarizes the subject matter. Section 3 and 4 describe the modelling software we use, and its support functions. Section 5 presents the reference model, with subsections for each mechanism from the full system. Section 6 concludes the paper.

2 Clock genes

The circadian clock regulates the 24-hour rhythm of biological processes such as sleep, hormone production, and metabolism. The central clock resides in the suprachiasmatic nucleus (SCN), a small region in the hypothalamus. This clock is driven by a core set of clock genes, including CLOCK, BMAL1, PER, and CRY, which interact in a transcriptional–translational feedback loop to maintain rhythmic gene expression across the day [12].

The CLOCK:BMAL1 dimer functions as a transcription factor that binds to E-box elements in the promoter regions of target genes, notably PER and CRY. When CLOCK:BMAL1 activity increases, it enhances the transcription of these genes, leading to the production of mRNA. This mRNA is exported from the nucleus to the cytoplasm, where it is translated into PER and CRY proteins by ribosomes. mRNA molecules are short-lived and are degraded once their message has been used.

After synthesis, PER and CRY proteins accumulate in the cytoplasm, form a complex, and translocate back into the nucleus. There, the PER:CRY complex inhibits CLOCK:BMAL1 activity by binding to the dimer and altering its structure, preventing further transcription of PER and CRY. This establishes a negative feedback loop that is essential for maintaining the circadian rhythm. PER and CRY proteins are also degraded over time through ubiquitin-mediated proteasomal pathways. This degradation relieves the inhibition on CLOCK:BMAL1, allowing a new cycle to begin.

Beyond regulating PER and CRY, CLOCK:BMAL1 also controls other genes, including AVP, which encodes vasopressin, a hormone involved in water balance and blood pressure regulation. This highlights how the molecular clock connects gene regulation to broader physiological functions.

3 Qualitative reasoning with DynaLearn

DynaLearn (https://www.dynalearn.nl) is an interactive tool that allows users to create and simulate qualitative models. The following ingredients are available to create representations.

Entities can be used for representing physical objects and/or abstract concepts that make up the system. *Configurations* can be used for representing structural relationships between entities.

Quantities can be used for representing changeable and measurable features of entities. Quantities have *Direction of change* (∂) (decreasing, steady, and increasing) and a *Quantity space* (a set of alternating point and interval values that the quantity can take on).

Causal dependencies can be used for representing directed relationships between quantities.

Correspondences can be used for representing co-occurring values and co-occurring directions of change.

In/equalities can be used for representing order information among values and among directions of change.

When simulating, *Initial values* are defined for quantities, typically (but not exclusively) at the start of *Causal paths* (sequences of causal dependencies). This can be a direction of change, an initial value or an *Exogenous* behaviour. Additionally, in/equalities can be specified.

The simulation produces a *State-graph*, which consist of one or more *States* (unique qualitative behaviour of the system) and *Transitions* (continuous passage) between pairs of states. The system behaviour throughout the state-graph can be inspected using the *Value-history* and the *Inequality-history*.

Working with DynaLearn can be done at different levels of complexity [13]. The reference model presented in this paper is situated at level 4. This level includes the causal dependencies influence (I+/I-) and proportionality (P+/P-) [14]. Learners can thus focus on the distinction between processes (I) (initial causes) and the propagation (P) of these through the system. Positive and negative feedback loops are also available and in/equality statements (< ≤ = ≥ >) can be used to represent the relative impact of competing processes.

4 Support functions in DynaLearn

Learning by creating a qualitative model can be an effective approach, but it requires appropriate guidance [15,16]. In the Denker project (https://denker.nu/) we have developed various forms of support that provide students with feedback to help them work more independently [3,4], while at the same time reducing the burden on the teacher [17]. Below we summarize our latest developments, seen from the student's perspective.

Norm-based advice (Fig. 1). Each modelling action performed by the student is compared to a reference model. When discrepancies occur a red question mark appears. Selecting it provides advice regarding possible mistakes. The student still has to come up with a solution.

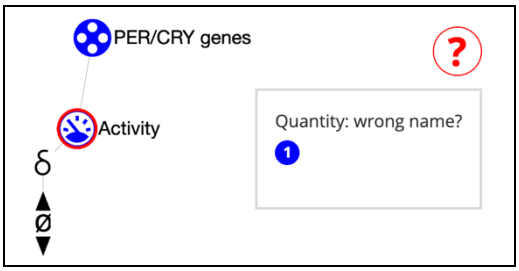

Fig. 1. Norm-based advice.

Scenario advice (Fig. 2). Each time a simulation is run, the software checks for missing information and inconsistencies given to the qualitative reasoning engine. When errors occur a blue exclamation mark appears that can be consulted for support. The example shown Fig. 2 reports a missing initial change. Scenario advice also identifies and highlights feedback loops.

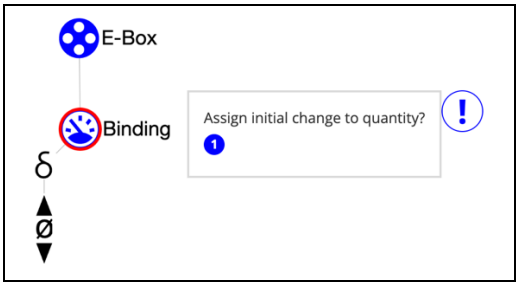

Fig. 2. Scenario advice.

Progress bar (Fig 3). This feature provides students a reflective overview of their progress. For each ingredient type the progress informs the students about (*i*) how many ingredients need to be created (e.g., 8 entities and 11 quantities), (*ii*) how many ingredients have already been created correctly (e.g., 8 entities and 4 quantities), and (*iii*) how many have been created incorrectly (e.g., 0 entities and 1 quantity). When all ingredients are correctly created, the numbers become green, while the number of incorrect ingredients is show in red.

Fig. 3. Progress bar (showing a selection to maintain readability).

Video function. For each ingredient type the software provides access to a short video that shows how ingredients of that type can be created.

Workbook. A lesson with the DynaLearn software is usually accompanied by a workbook. This workbook provides short textual and visual explanations of the phenomenon discussed, as well as short explanations of the

ingredients that form the qualitative vocabulary. The workbook is built up from a series of successive units and support the students in a stepwise approach to the modelling assignment (see https://dynalearn.nl/#lessons for examples).

Evaluation studies with students in regular classroom settings (cf. [18, 19]) show that students are able to work independently with the above-described approach.

5 The Model

We first created the full model, including evaluation and refinement with domain experts. The final result of that effort is shown in Fig. 17. Simulating this model delivers a sequence of eight consecutive states, which together form the oscillating behaviour of the circadian clock [11]. Table 7 shows for each quantity its value (v) and direction of change ∂ in each of the states. Note that the model emphasises the direction of change for each quantity, while ignoring specific values. Hence, all the values in Table 7 are unknown (u) (see next sections for further details). Although specific values are unknown, there is still information about the strength of competing processes. As such, Table 8 shows the balance for two pairs of competing processes across the state-graph using in/equality statements.

After the model was completed, it was decomposed into six units facilitating a stepwise modelling and learning process for students. The lesson begins with thinking about how increasing CLOCK:BMAL1 activity enhances the transcription of the PER and CRY genes through binding to the E-box. Next, the lesson explores how mRNA production and degradation, two opposing processes, regulate mRNA levels. This is followed by modelling translation at the ribosomes, where the PER and CRY proteins are synthesized and subsequently degraded, again illustrating competing regulatory processes. The lesson than moves on to how PER and CRY proteins form a complex that is translocated to the nucleus, inhibiting CLOCK:BMAL1 binding and thereby establishing a negative feedback loop. The lesson ends by exploring how CLOCK:BMAL1 also regulates the AVP gene, linking clock genes to broader physiological processes.

The next sections discuss these units. Each section shows the information provided to the students in the accompanying workbook, discusses the reason underpinning the content of the specific unit as well as their order of appearance with respect to other units, and explains the part of the model that students are expected to create in the unit.

5.1 CLOCK:BMAL1 stimulates transcription

The workbook starts by reactivating students' awareness of the location of the hypothalamus and the suprachiasmatic nucleus (SCN) in the brain using a visual (Fig. 4) and a textual explanation, notably: *The circadian clock regulates the 24-hour rhythm of biological processes in our body, such as sleep, hormone production and metabolism. The central circadian clock is located in the suprachiasmatic nucleus (SCN), a small area in the hypothalamus. This clock is driven by clock genes, such as CLOCK, BMAL1, PER and CRY. These genes work together in a feedback loop to regulate the rhythmic expression of genes involved in important physiological processes.*

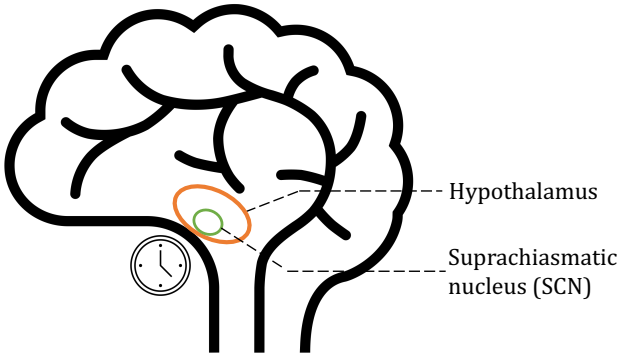

Fig. 4. Position of the hypothalamus and the suprachiasmatic nucleus in the brain.

The workbook continuous with introducing the first unit. Again, a visual is used (Fig. 5) accompanied by text: *CLOCK:BMAL1 is a dimer of two proteins. When the activity of CLOCK:BMAL1 increases, CLOCK:BMAL1 binds to a greater extent to the E-box of the PER and CRY genes, which leads to increased transcription of these genes.*

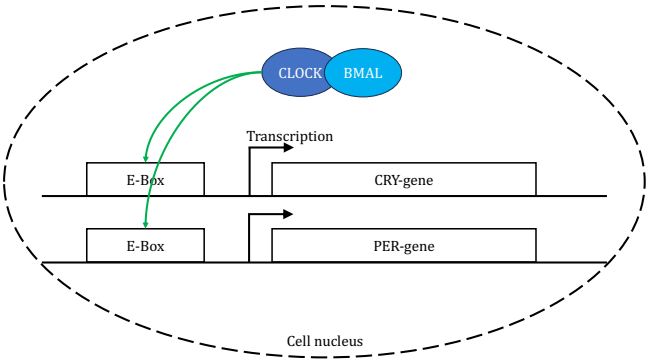

Fig. 5. Illustration of how CLOCK:BMAL1 binds to the E-box of the PER and CRY and affects the transcription of these genes.

The workbook instructs students to create the four entities involved, link them using three configurations, and add the three quantities. Next, students are challenged to figure out the causal dependencies, both between *which* quantities and *which* type (i.e., P or I), and create these in the model. After also creating the exogenous influence students simulate the model and inspect the simulation results. Fig. 6 shows the model including the simulation results. To consolidate their understanding, students answer a close question in the workbook about directions of change and causal propagation (see Table 1).

Table 1. Close question at the end of the first unit. Students must select the correct options among the italic alternatives.

In state 1, the activity of CLOCK:BMAL1 increases. As a result, the binding to the E-box will *decrease/remain the same/increase* and the transcription of the PER/CRY genes will *decrease/remain the same/increase*.

Being a level 4 model, students are expected to have created models at lower levels before. Still, the workbook takes a self-contained approach so that also students not familiar with the software can take the lesson. Hence, the workbook has short explanatory texts describing each of the ingredient types used in the model. It also provides pointers to the various videos available in the software that illustrate how to create each of the ingredient types.

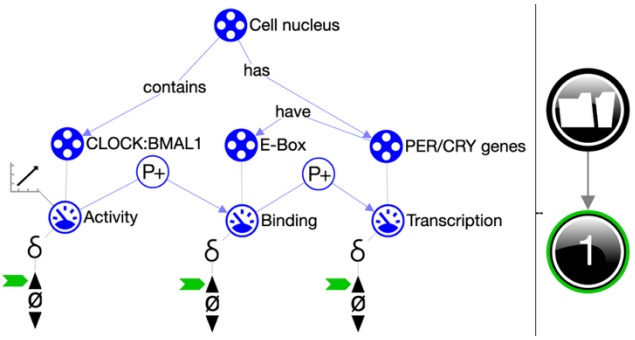

Fig. 6. Model of how increased CLOCK:BMAL1 activity leads to increased transcription of the PER and CRY genes.

From a model or modelling perspective there are no strong arguments why the lesson should start with the unit described above. However, from a content point of view the activity of CLOCK:BMAL1 is typically regarded the key factor driving the circadian clock phenomenon. Simultaneously, the unit does introduce a range of basic ingredients in an easy to master setting (including, entity, quantity, proportionality, derivate, exogenous), and hence a good starting point.

5.2 Transcription of PER and CRY genes

For the next unit in the lesson, it makes sense to extend the causal path, either before or following the details created in the previous unit. 'Following' is preferred for two reasons. First, it introduces a new systems thinking concept, namely the notion of a process. Second, the part 'before' is the result of a feedback loop. Understanding the content (reason) of that feedback loop makes much more sense when the details leading to it have been processed. Hence, the focus in this unit is on the next step in the causal chain.

The workbook introduces the content of this unit using a visual (Fig. 7) and a short textual explanation: *When CLOCK:BMAL1 is bound to the E-box of the PER and CRY genes the transcription is activated, leading to the production of mRNA.*

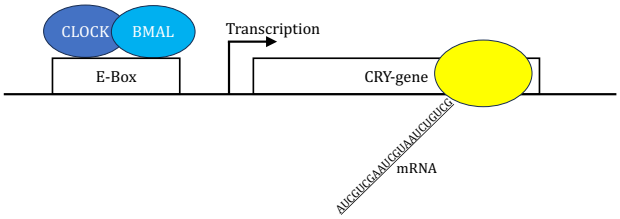

Fig. 7. Illustration of transcription of the CRY gene.

The workbook briefly explains the notion of processes as the causes of change and how that differs from propagating changes. Next, the students are instructed to create the required model ingredients, notably the quantity *[mRNA PER/CRY]*, the quantity space for *Transition*, and the influence from *Transition* on *[mRNA PER/CRY]*. The results are shown in Fig. 8.

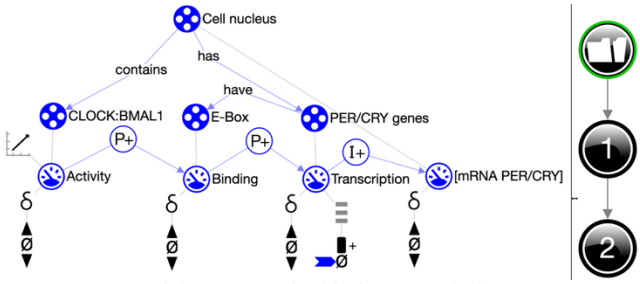

Fig. 8. Model augmented with the transcription process.

To further foster the understanding of how processes work, the students run at least two distinct scenarios. The simulation results shown in Table 2, start with *Activity* being steady (∂=0), *Transcription* being active (v=+), and the rest of the information about the quantities set to unknown. This results in a single state, in which all quantities are steady, except for *[mRNA PER/CRY]* which increases due to *Transcription* being active. This simulation thus shows that although *Transcription* is steady (∂=0) it still causes the *[mRNA PER/CRY]* to increase (∂>0), because *Transcription* is a process.

Table 2. Simulation results for the model shown in Fig. 8, with transcription starting at >∅.

Quantity	Initial	S_1
Activity	<u, 0>	<u, 0>
Binding	<u, u>	<u, 0>
Transcription	<+, u>	<+, 0>
[mRNA PER/CRY]	<u, u>	<u, +>

The simulation results shown in Table 3 take a different starting point. Now *Activity* is increasing (∂>0), *Transition* is inactive (v=0), and the rest of the information is unknown. This makes the system go through two states. In the first state (S_1), all quantities are increasing, except for *[mRNA PER/CRY]* because the *Transition* process is inactive (v=0). In the next state (S_2), *Transition* becomes active (V>0) and hence *[mRNA PER/CRY]* is now also increasing.

Table 3. Simulation results for model shown in Fig. 8, with transcription starting at zero (∅).

Quantity	Initial	S_1	S_2
Activity	<u, +>	<u, +>	<u, +>
Binding	<u, u>	<u, +>	<u, +>
Transcription	<0, u>	<0, +>	<+, +>
[mRNA PER/CRY]	<u, u>	<u, 0>	<u, +>

5.3 Transport and degradation of mRNA

The lesson moves forward following the causal path. In addition to this being a logical step from the content point of view, it also deepens the systems thinking experience because

this unit introduces a second and competing process on the production of *[mRNA PER/CRY]*.

The workbook starts with a visual (Fig. 9), and a short textual explanation: *mRNA is transported from the cell nucleus to the cytoplasm, where the translation takes place at the ribosomes. mRNA has a limited lifespan and is degraded once it has completed its task: delivering the code for translation*.

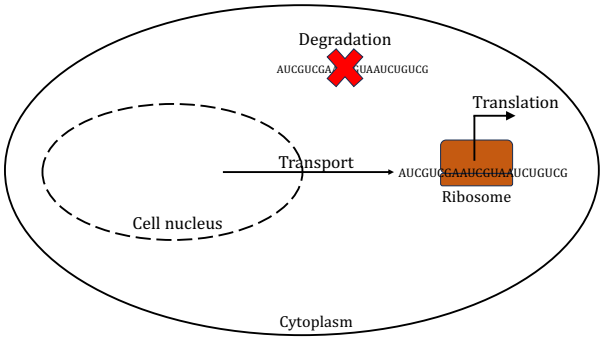

Fig. 9. Illustration of transport and degradation of mRNA.

The building proceeds in three steps. First, the entity *Ribosomes* is added, with quantity *Translation* and how that relates to quantity *[mRNA PER/CRY]*. This part is simulated, and students answer a close question to consolidate their understanding. Second, the degradation process is added, active in the *Cytoplasm* and removing the *[mRNA PER/CRY]*. As the latter is competing with the *Transcription* process the students are informed about specifying the relative impact using an in/equality statement. This part is next simulated with *Transcription* < *Degradation mRNA* resulting in a single state in which the production of *[mRNA PER/CRY]* decreases. This step also ends with a close question. The third part emphasizes the point that the degradation depends on the amount of mRNA. Hence, a proportionality is required from *[mRNA PER/CRY]* to *Degradation mRNA* which positively affects the degradation process. Fig 10 shows the resulting model, including all the details added in this unit.

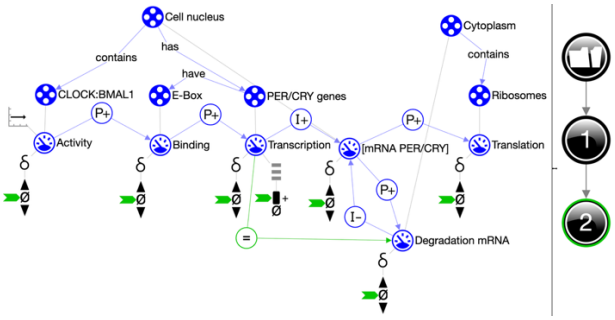

Fig. 10. Model augmented with mRNA transport and degradation.

The model constructed so far is then simulated. The results are shown in Table 4. The unit ends with two close questions for the student to answer in the workbook.

Table 4. Simulation results for model shown in Fig. 10 (T refers to *Transcription* and D refers to *Degradation mRNA*).

Quantity	Initial	S_1	S_2
Activity	<u, 0>	<u, 0>	<u, 0>
Binding	<u, u>	<u, 0>	<u, 0>
Transcription	<+, u>	<+, 0>	<+, 0>
[mRNA PER/CRY]	<u, u>	<u, ->	<u, 0>
Translation	<u, u>	<u, ->	<+, 0>
Degradation mRNA	<u, u>	<u, ->	<u, 0>
T ? D	T < D	T < D	T = D

5.4 Synthesis and degradation of PER and CRY

At this stage in the lesson, there is one more step in the causal path before the feedback loop can be closed. Hence, the fourth unit is about this part: the synthesis and degradation of PER and CRY proteins. As the students are expected to have become familiar with most of the modelling actions, the complexity can be increased. Hence, this unit challenges the student to complete all the required modelling actions before moving towards the simulation.

The workbook keeps the same routine, by presenting a visual (Fig. 11) and a short textual explanation: *The translation to the ribosomes ensures the production of the proteins CRY and PER. After their syntheses in the cytoplasm, these proteins are broken down again over time.*

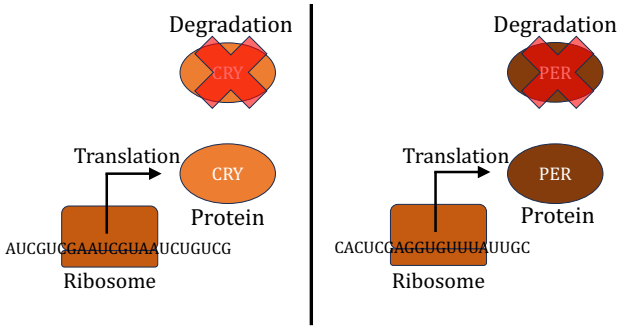

Fig. 11. Illustration of the synthesis and degradation of PER and CRY proteins.

As mentioned above, students are challenged to build this unit in one go. First, they must add the two new quantities: *[protein PER/CRY]* and *Degradation protein*, both belonging to the entity *Cytoplasm*. Next, they must create the two processes: *Translation* with a positive and *Degradation protein* with a negative impact on the production of the proteins. The relative strength of the processes must be specified with an in/equality. The workbook suggests the students to use: *Translation* > *Degradation protein.* Finally, changes in the degradation process depend on changes in the amount of proteins, hence a positive proportionally must be added. Fig. 12 show the part of the model created in this unit.

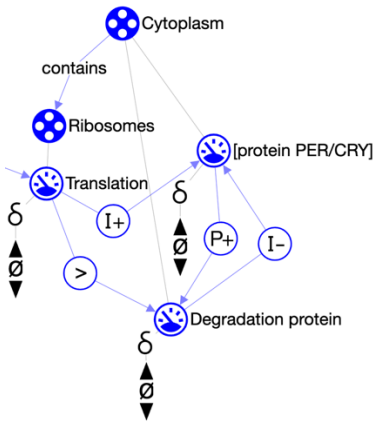

Fig. 12. Model augmented with the synthesis and degradation of the PER and CRY proteins (Fig. 10 shows the preceding causal path in the already created part of the model).

The workbook instructs the students to start the simulation with *Transcription = Degradation mRNA* (and *Translation > Degradation protein*, as mentioned above) while keeping all other initial settings as in the previous unit. With these settings the simulation results in a state-graph consisting of two states in which the balance between *Translation* and *Degradation protein* moves from unequal (S_1) to equal (S_2), while the rest of the quantities (created in the previous units) remain steady.

5.5 Feedback from PER and CRY proteins on CLOCK:BMAL1

With the key processes in place the causal path can now be closed, creating the feedback loop. The modelling effort in this unit appears relatively simple (creating one quantity and two proportional relationships), yet the impact on the simulation results is relatively large, requiring thoughtful handling and reasoning.

The workbook starts with a visual (Fig. 13) and the accompanying explanation: *After their synthesis in the cytoplasm, PER and CRY form a complex that moves to the cell nucleus. At its core, this complex binds directly to the CLOCK:BMAL1 dimer, changing the structure of the dimer. As a result, CLOCK:BMAL1 can no longer effectively bind to the E-box elements of the DNA promoters of target genes, such as PER and CRY. This creates a negative feedback loop in the circadian clock genes.*

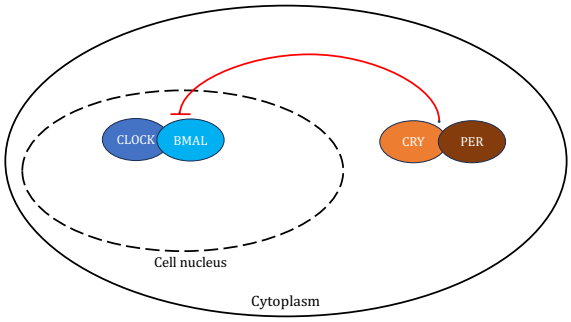

Fig. 13. Illustration of the feedback from PER and CRY proteins on CLOCK:BMAL1.

The workbook instructs the student to first create the quantity *[protein PER/CRY]* belonging to the entity *Cell nucleus*. Next, to create a positive proportionality between the *[protein PER/CRY]* in the *Cytoplasm* and the *[protein PER/CRY]* in the *Cell nucleus*, to represent the movement of the proteins to the nucleus. Next, to create a negative proportionality from the latter to the quantity *Activity* of the *CLOCK:BMAL1* entity, to represent the negative impact of the proteins. See Fig 17 for details (while ignoring the APV gene part, which belongs to the next unit).

Because the feedback loop now determines the behaviour of the CLOCK:BMAL1 activity, the exogenous influence on quantity *Activity* should be removed (compare e.g. Fig. 10 with Fig. 17 for details).

To focus the simulation on the essence of the systems' behaviour, the cycle of the circadian clock, the workbook suggests removing the quantity space previously added to the quantity *Transcription* of the entity *PER/CRY genes*. Earlier in the lesson this quantity space was needed to learn students about the working of processes (see e.g. Fig. 8). However, the transcription process is now exclusively competing with the degradation process and their mutual relationship is captured using an in/equality. Hence, the quantity space as such has become superfluous.

The initial settings required for running the simulation are (also shown in Fig. 17):

- *Transcription = Degradation mRNA*
- *Translation > Degradation protein*

This produces a state-graph with eight consecutives states which together implement the cyclic behaviour (Fig. 17, right-hand side). But how to see the characteristic behaviour of the key quantities involved? To address this, students are informed about the *value history* option in the software. Specifically, they are instructed to align and investigate the histories for the quantities *[protein PER/CRY]* of *Cell nucleus* and *Activity* of *CLOCK:BMAL1* (shown in Fig. 14).

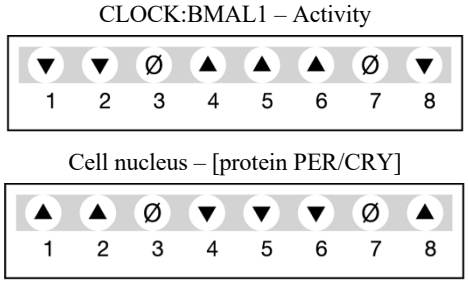

Fig. 14. Value histories for [protein PER/CRY] in cell nucleus and the CLOCK:BMAL1 activity.

To complete what has been learned in this unit, the workbook gives the students an assignment to draw a graph (shown Table 5 and Fig. 15).

Table 5. Graph drawing assignment for students.

Draw the graph showing the activity of CLOCK:BMAL1 and [protein PER/CRY] over two cycles. Use the value history and the following information:

- The CLOCK:BMAL1 dimer is most active at the beginning of the day (just after the biological night).
- Around this time, the inhibitory effect of PER and CRY on CLOCK:BMAL1 is minimal, because PER and CRY proteins have broken down during the night.
- This increased activity stimulates the transcription of PER, CRY, and other clock genes, which restarts the cycle.

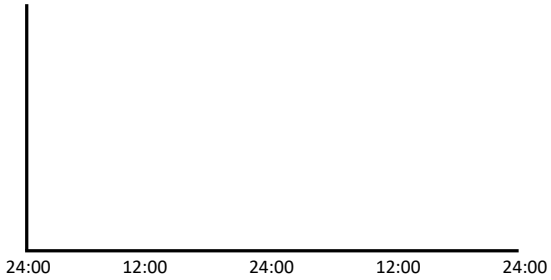

Fig. 15. Graph drawing assignment for students.

5.6 CLOCK:BMAL1 and vasopressin

The lesson ends by exploring how CLOCK:BMAL1 also regulates the AVP gene, linking clock genes to broader physiological processes. The workbook provides the following visual (Fig. 16) and explanatory text: *The CLOCK:BMAL1 dimer also regulates the AVP gene encoding vasopressin, an important hormone involved in the regulation of water balance and blood pressure.*

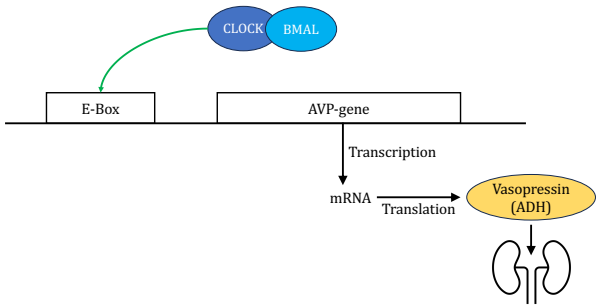

Fig. 16. Illustration of the relationship between CLOCK:BMAL1 and the AVP gene and the effect of the latter on vasopressin.

This time the workbook gives only general instructions, such as *Finalize the model by creating the entities (2), configurations (1), quantities (2), and relationships (2) with which CLOCK:BMAL1 regulates the transcription of the AVP gene.* The modelling results can be found in Fig. 17 (see ingredients related to the *EVP gene* and its *E-Box*).

Using the simulation results, the students answer the final close question in this unit focusing on S_1 of the cyclic behaviour (shown in Table 6).

Table 6. Close question at the end of the last unit. Students must select the correct options among the italic alternatives.

In state 1, the CLOCK:BMAL1 activity *decreases/remains the same/increases*. As a result, the binding to the E-box of the AVP gene will *decrease/remain the same/increase* and the transcription of the AVP gene will *decrease/remain the same/increase*.

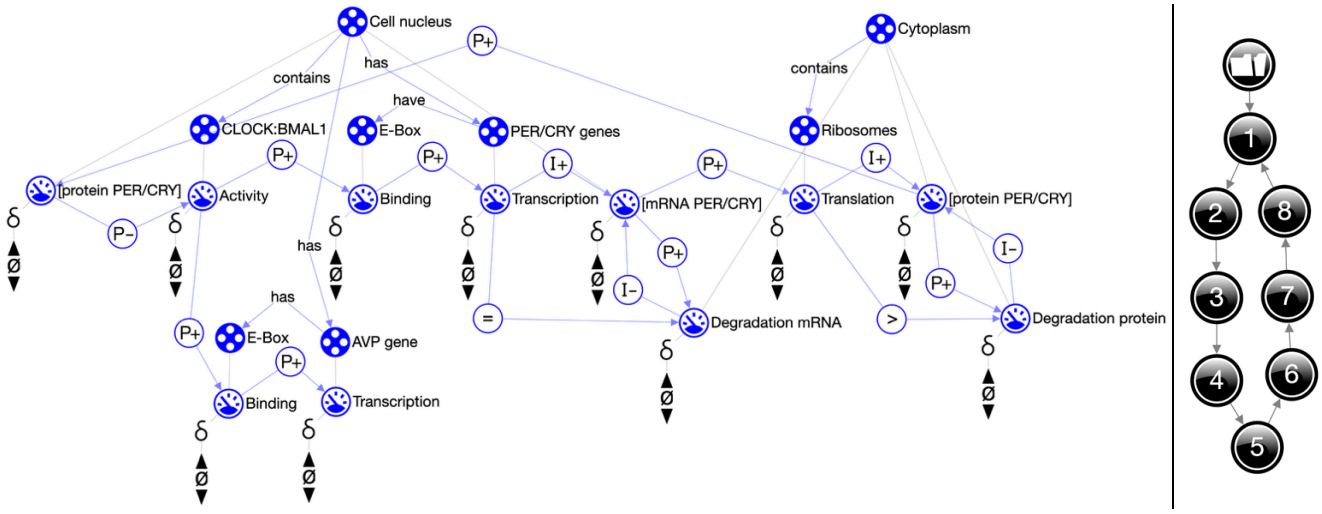

Figure 17. Qualitative model of clock genes. Left-hand side shows the representation. Right-hand side shows the simulation results as a state-graph which consists of a loop of 8 consecutive states (1 → 2 → 3 → 4 → 5 → 6 → 7 → 8 → 1 etc.).

Table 7. Simulation results for the clock genes model shown in Fig. 17. S refers to State, <v, ∂> refers to value and derivative (change), respectively, and u refers to unspecified value.

Entity	Quantity	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Cell nucleus	[protein PER/CRY]	<u, +>	<u, +>	<u, 0>	<u, ->	<u, ->	<u, ->	<u, 0>	<u, +>
	[mRNA PER/CRY]	<u, 0>	<u, ->	<u, ->	<u, ->	<u, 0>	<u, +>	<u, +>	<u, +>
CLOCK:BMAL1	Activity	<u, ->	<u, ->	<u, 0>	<u, +>	<u, +>	<u, +>	<u, 0>	<+, ->
E-Box (PER/CRY genes)	Binding	<u, ->	<u, ->	<u, 0>	<u, +>	<u, +>	<u, +>	<u, 0>	<+, ->
PER/CRY genes	Transcription	<u, ->	<u, ->	<u, 0>	<u, +>	<u, +>	<u, +>	<u, 0>	<+, ->
Ribosomes	Translation	<u, 0>	<u, ->	<u, ->	<u, ->	<u, 0>	<u, +>	<u, +>	<u, +>
Cytoplasm	Degradation mRNA	<u, 0>	<u, ->	<u, ->	<u, ->	<u, 0>	<u, +>	<u, +>	<u, +>
	[protein PER/CRY]	<u, +>	<u, +>	<u, 0>	<u, ->	<u, ->	<u, ->	<u, 0>	<u, +>
	Degradation protein	<u, +>	<u, +>	<u, 0>	<u, ->	<u, ->	<u, ->	<u, 0>	<u, +>
E-Box (AVP gene)	Binding	<u, ->	<u, ->	<u, 0>	<u, +>	<u, +>	<u, +>	<u, 0>	<+, ->
AVP gene	Transcription	<u, ->	<u, ->	<u, 0>	<u, +>	<u, +>	<u, +>	<u, 0>	<+, ->

Table 8. Simulation results continued, showing the inequality information for two quantity pairs in each of the states.

Compared quantities	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
PER/CRY genes: Transcription *versus* Cytoplasm: Degradation mRNA	=	<	<	<	=	>	>	>
Ribosomes: Translation *versus* Cytoplasm: Degradation protein	>	>	=	<	<	<	=	>

6 Conclusion and Discussion

This paper presents a qualitative model of the circadian clock cycle. It provides an explicit representation of the dynamic system in which the clock genes, notably CLOCK, BMAL1, PER and CRY work together in a feedback loop to regulate the rhythmic expression of genes involved in important physiological processes.

After the model was established, and reviewed by experts, it was decomposed into a sequence of six units that aids students in a stepwise modelling endeavour in which they re-create the qualitative model and thereby learn about this phenomenon.

The results presented in this paper augment our earlier work on melatonin regulation [10]. In that model the clock cycle was represented as an exogenous influence, because the emphasis of the lesson was on the consequences of this cycle behaviour. Capturing the clock phenomenon itself in a model significantly deepens the available learning material, as well as our knowledge on how to create such complex models.

The kernel of the model consists of the two consecutive pairs of competing processes. First, the transcription and degradation of the messenger RNA for the PER and CRY genes in the cell nucleus. Second, the translation and degradation of the PER and CRY proteins in the cytoplasm. This mechanism is stimulated by activity of the CLOCK:BMAL1 dimer and inhibited via a negative feedback loop when the proteins are translocated back to the cell nucleus.

The model has been peer-reviewed by experts in the field of the circadian clock. Evaluation with students is planned for future work. Earlier evaluation studies with students have shown that students are well capable or self-regulated learning with the lessons and supporting instruments as presented in this paper (cf. [18]).

Acknowledgments

The research presented here is part of the BioClock Consortium which is funded by the NWA-ORC programme of the Dutch Research Council (NWO), project number 1292.19.077, https://bioclockconsortium.org/. We would like to thank Ines Machado, Andries Kalsbeek and other co-workers for their constructive feedback and support.

7 References

[1] Bredeweg, B. & Forbus. K.D (2016). Qualitative Representations for Education. In: R. A. Sottilare, A. C. Graesser, X. Hu, A. M. Olney, B. D. Nye, & A. M. Sinatra (Eds.), *Design Recommendations for Intelligent Tutoring Systems: Domain Modeling*, 4, 55-68.

[2] Jochem Liem, J. (2013). Supporting conceptual modelling of dynamic systems: A knowledge engineering perspective on qualitative reasoning. PhD thesis, University of Amsterdam, The Netherlands.

[3] Bredeweg, B., Kragten, M., Holt, J., Kruit, P., van Eijck, T., Pijls, M., Bouwer, A., Sprinkhuizen, M., Jaspar, E., & de Boer, M. (2023). Learning with Interactive Knowledge Representations. Applied Sciences, 13(9), Article 5256.

[4] Bredeweg, B., Kragten, M., & Spitz, L. (2021). Qualitative Representations for Systems Thinking in Secondary Education. Paper presented at 34th International Workshop on Qualitative Reasoning, Montreal, Canada.

[5] Spitz, L., Kragten, M., & Bredeweg, B. (2021). Learning Domain Knowledge and Systems Thinking using Qualitative Representations in Secondary

Education (grade 8-9). 34th International Workshop on Qualitative Reasoning, Montreal, Canada.

[6] Kragten, M., Spitz, L., & Bredeweg, B. (2021). Learning Domain Knowledge and Systems Thinking using Qualitative Representations in Secondary Education (grade 9-10). 34th International Workshop on Qualitative Reasoning, Montreal, Canada.

[7] Kragten, M., Jaspar, E. J. O. A., & Bredeweg, B. (2022). Learning Domain Knowledge and Systems Thinking using Qualitative Representations in Secondary Education (grade 10-12). 35th International Workshop on Qualitative Reasoning, Vienna, Austria.

[8] Kragten, M., Hoogma, T., & Bredeweg, B. (2023). Learning domain knowledge and systems thinking using qualitative representations in upper secondary and higher education. 36th International Workshop on Qualitative Reasoning, Krakow, Poland.

[9] Kragten, M., & Bredeweg, B. (2023). Describing the characteristics of circular and elliptical motion using qualitative representations. 36th International Workshop on Qualitative Reasoning, Krakow, Poland.

[10] Fawzi, N., Kragten, M., & Bredeweg, B. (2024). Qualitative Reference Model for Learning about Melatonin Regulation. 37th International Workshop on Qualitative Reasoning, Santiago de Compostella, Spain.

[11] Kumar, V. (ed.). (2017). Biological Timekeeping: Clocks, Rhythms and Behaviour. Springer, Delhi, India.

[12] Partch, C. L., Green, C. B., & Takahashi, J. S. (2014). Molecular architecture of the mammalian circadian clock. Trends in Cell Biology, 24(2), 90–99.

[13] Bredeweg, B., Liem, L., Beek, W., Salles, P. & Linnebank, F. (2010). Learning spaces as representational scaffolds for learning conceptual knowledge of system behaviour. In: M. Wolpers, P. A. Kirschner, M. Scheffel, S. Lindstaedt, & V. Dimitrova (Eds.), *Technology Enhanced Learning*, LNCS 6383, 46-61. Springer, Cham.

[14] Forbus, K.D. (2018). Qualitative representations. How people reason and learn about the continuous world. Cambridge, Massachusetts: The MIT Press.

[15] Bredeweg, B., Liem, J., Beek, W., Linnebank, F., Gracia, J., Lozano, E., Wißner, M., Bühling, R., Salles, P., Noble, R., Zitek, A., Borisova, P. & Mioduser, D. (2013). DynaLearn - An intelligent learning environment for learning conceptual knowledge. *AI Magazine*, 34(4), 46-65.

[16] Gautam Biswas, G., Segedy J.R. & Bunchongchit, K. (2016). From Design to Implementation to Practice a Learning by Teaching System: Betty's Brain. *International Journal of Artificial Intelligence in Education*, 26, 350-364.

[17] Kragten, M., Hoogma, T. E., & Bredeweg, B. (2024). Integration of a Teacher Dashboard in a Hybrid Support Approach for Constructing Qualitative Representations. In: R. Ferreira Mello, N. Rummel, I. Jivet, G. Pishtari, & J. A. Ruipérez Valiente (Eds.), Technology Enhanced Learning, LNCS 15159, 208-221. Springer, Cham.

[18] Marco Kragten, M. & Bredeweg, B. (2024). Calcium Regulation Assignment: Alternative Styles in Successfully Learning about Biological Mechanisms. In: Artificial Intelligence in Education, LNAI 14829, 220-234. Springer, Cham.

[19] Kragten, M., & Bredeweg, B. (2024). The effectiveness of lightweight automated support for learning about dynamic systems with qualitative representations. 39th ACM/SIGAPP Symposium on Applied Computing, 11-20.