Temporal Logic Patterns for Querying Dynamic Models of Cellular Interaction Networks
Pedro T. Monteiro, Delphine Ropers, Radu Mateescu, Ana T. Freitas, Hidde de Jong

To cite this version:

HAL Id: inria-00357805
https://inria.hal.science/inria-00357805
Submitted on 2 Feb 2009

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Temporal Logic Patterns for Querying Dynamic Models of Cellular Interaction Networks

Pedro T. Monteiro\textsuperscript{1,2,3}, Delphine Ropers\textsuperscript{1}, Radu Mateescu\textsuperscript{1}, Ana T. Freitas\textsuperscript{2}, Hidde de Jong\textsuperscript{1,*}

\textsuperscript{1}INRIA Grenoble - Rhône-Alpes, 655 Av. de l’Europe, Montbonnot, 38334 St. Ismier Cedex, France
\textsuperscript{2}IST/INESC-ID, 9 Rua Alves Redol, 1000-029 Lisbon, Portugal
\textsuperscript{3}Université Claude Bernard Lyon 1, 43 Bvd. du 11 Nov. 1918, 69622, Villeurbanne Cedex, France

Associate Editor: XXXXXXX
Received on XXXXX; revised on XXXXX; accepted on XXXXX

ABSTRACT

Motivation: Models of the dynamics of cellular interaction networks have become increasingly larger in recent years. Formal verification based on model checking provides a powerful technology to keep up with this increase in scale and complexity. The application of model-checking approaches is hampered, however, by the difficulty for non-expert users to formulate appropriate questions in temporal logic.

Results: In order to deal with this problem, we propose the use of patterns, that is, high-level query templates that capture recurring biological questions and that can be automatically translated into temporal logic. The applicability of the developed set of patterns has been investigated by the analysis of an extended model of the network of global regulators controlling the carbon starvation response in \textit{Escherichia coli}.

Availability: GNA and the model of the carbon starvation response network are available at http://www-helix.inrialpes.fr/gna

Contact: Hidde.de-Jong@inrialpes.fr

1 INTRODUCTION

Models of the dynamics of cellular interaction networks have become increasingly larger in recent years. While whole-cell models are not on the horizon yet, complex networks underlying specific cellular processes have been modeled in detail, such as the osmotic shock response in yeast (Klipp et al., 2005), the yeast cell cycle (Chen et al., 2004), and signalling pathways involved in cancer (Schoeberl et al., 2002). The study of these models by means of analysis and simulation tools leads to large amounts of predictions, typically time-courses of the concentrations of several dozens of molecular components in a variety of physiological conditions and genetic backgrounds. This raises the question how to make sense of these simulation, that is, how to obtain an understanding of the way in which particular molecular mechanisms control the cellular process under study, and how to identify interesting predictions of novel phenomena that can be confronted with experimental data.

Methods from the field of formal verification provide a promising way to deal with the analysis of large and complex models of cellular interaction networks (Fisher et al., 2007). Generally speaking, formal verification proceeds by specifying dynamical properties of interest as statements in temporal logic. Efficient so-called model-checking algorithms, implemented in publicly-available computer tools, exist to determine whether the statements are true or false, and thus whether the dynamic properties are satisfied by the model. The methods are generally applicable to discrete models of cellular interaction networks, or continuous models that have been discretized under a suitable abstraction criterion. Several examples exist of the application of model-checking approaches in systems biology (e.g., Antoniotti et al., 2003; Batt et al., 2005; Bernot et al., 2004; Calder et al., 2005; Chabrier-Rivier et al., 2004; Fisher et al., 2007).

Formal verification based on model checking provides a powerful technology to query models of cellular interaction networks. It raises a number of new issues though, notably that of formulating good questions when analyzing a huge network model. The problem of posing relevant and interesting questions is critical in modeling in general, but even more so in the context of applying formal verification techniques, due to the fact that it is not easy for non-experts to formulate queries in temporal logic. For instance, the property "Gene $g$ is eventually expressed, and necessarily preceded over the whole duration of the experiment by a concentration larger than 0.9 $\mu$M of the transcription factor $P$" corresponds to the following CTL formula, where $\phi_g$ denotes expression of gene $g$:

\[ EF(\phi_g) \land \neg E(T(\exists U(\neg([P] > 0.9 \mu M) \land E(\exists U \phi_g)))) \]

The response to this problem proposed by the formal verification community is the use of patterns, that is, high-level query templates that capture recurring questions in a specific application domain and that can be automatically translated to temporal logic (Dwyer et al., 1999). Apart from lists of example queries (Chabrier-Rivier et al., 2004), the systematic definition of queries has not received any attention in systems biology thus far.

The aim of this paper is to develop a set of patterns for the analysis of dynamic models of cellular interaction networks. Its main contributions lie, first, in the development of generic query templates, based on a review of frequently-asked questions by modelers, and the translation of these templates into temporal logic formulas (Sec. 2). Second, we apply the patterns for analyzing the qualitative dynamics of a large and complex model of the \textit{E. coli}...
carbon starvation response (Sec. 3). This model extends a previous model (Ropers et al., 2006) by taking into account additional regulators of the bacterium, notably a module centered around the general stress response factor RpoS. We verify the control the latter is predicted to exert on the DNA supercoiling level in the cell.

2 PATTERNS OF BIOLOGICAL QUERIES

2.1 Description of network dynamics

As a basic hypothesis, we assume that the dynamics of cellular interaction networks can be modeled by means of finite state transition systems (FSTSs) (Clarke et al., 1999). The latter formalism provides a general description of a dynamical system that implicitly or explicitly underlies many of the existing discrete formalisms used to model cellular interaction networks, such as Boolean networks and their generalizations, Petri nets, and process algebras. In addition, observed data time-series and continuous models of cellular interaction networks, by defining appropriate discrete abstractions, can also be mapped to FSTSs. The generality of the FSTS formalism is important for assuring the wide applicability of the patterns developed in this section. Moreover, statements in temporal logics are usually interpreted on FSTSs, so that the latter naturally connect network models to model-checking tools.

A finite state transition system is formally defined as a tuple \( \Sigma = (S, AP, T, S_0) \), where \( S \) is a set of states, \( AP \) is a set of atomic propositions, \( T : S \rightarrow 2^{AP} \) is a labeling function that associates to a state \( s \in S \) the set of atomic propositions satisfied by \( s \), \( T \subseteq S \times S \) is a relation defining transitions between states, and \( S_0 \subseteq S \) is a set of initial states. For our purpose, \( S \) describes the possible states of the cellular interaction network, each of which is characterized by a set of atomic propositions, such as that the concentration of protein \( P \) is increasing, or that the concentration of metabolite \( M \) is smaller than 5 mM.

2.2 Identification of patterns

The notion of patterns originates in architecture (Alexander et al., 1977) and was introduced in the domain of software engineering as a means to capture expert solutions to recurring problems in program design (Gamma et al., 1994). In the formal verification domain they have been introduced in an influential paper by Dwyer et al. (1999), to help non-expert users formulate their temporal-logic queries. In the latter context, patterns are high-level descriptions of frequently asked questions in an application domain that are formulated in structured natural language rather than temporal logic. The aim of the patterns is not to cover all possible questions an expert can think of, but rather to simplify the formulation of those that are primary.

The difficulty of proposing patterns is to come up with a limited number of query schemas that are sufficiently generic to be applicable in a variety of situations, and at the same time sufficiently concrete to be comprehensible for the non-expert user. Moreover, the overlap between the patterns should be minimal. We analyzed a large number of modeling studies in systems biology (starting from the references in Szallasi et al. (2006), as well as previous applications of model checking and temporal logic (e.g., Antoniotti et al., 2003; Batt et al., 2005; Bernot et al., 2004; Calder et al., 2005; Chabrier-Riviere et al., 2004; Fisher et al., 2007). This bibliographic research allowed us to identify an open-ended list of questions on the dynamics of genetic, metabolic, and signal transduction networks, for instance:

- Is the basal glycerol production level combined with rapid closure of Fps1 sufficient to explain an initial glycerol accumulation after osmotic shock? (Klipp et al., 2005)
- Once a cell has executed START, does it slip back into G1 phase and repeat START? Or rather, must it execute a FINISH to return to G1? (Chen et al., 2004).
- Does Shc phosphorylation exhibit a relative acceleration with decreasing EGF concentration and show a decline over time? (Schoeberl et al., 2002)

The identified questions were grouped into four categories, depending on whether they concerned the occurrence/exclusion, consequence, sequence, and invariance of cellular events. For each of these, we developed an appropriate pattern, capturing the essence of the question and the most relevant variants.

2.3 Description of patterns

The patterns consist of structured natural language phrases, represented in schematic form, with placeholders for so-called state descriptors. A state descriptor is a statement expressing a state property, and takes the form of (a Boolean combination of) atomic propositions. Let \( \phi, \psi \) be state descriptors, then

\[
\phi, \psi := p_1 \in AP | p_2 \in AP | \ldots
\]

\[
:= \neg \phi \land \psi \Rightarrow \psi | \ldots
\]

The state descriptors are interpreted on the FSTS, in the sense that their meaning is formally defined as the set of states \( S \) satisfying the state descriptor. In addition to (Boolean combinations of) atomic propositions, the state descriptors may be temporal-logic formulas defined on the atomic propositions. We will return to this generalization in Sec. 5.

It is often convenient to introduce predefined state descriptors that capture Boolean combinations of atomic propositions that are recurrently used. Some examples of predefined state descriptors that we found useful are the following:

- \( \text{Increases/Degreases} \) : the concentration of molecular component \( i \) increases/decreases in this state;
- \( \text{IsSteadyState} \) : the concentrations of all molecular components are steady in this state;
- \( \text{IsOscillatoryState} \) : the concentrations of some molecular components oscillate in this state.

Notice that the precise definition of the state descriptors depends on the particular type of FSTS that is used, as the latter determines the set of atomic propositions \( AP \).

**Definition 1** (Occurrence/exclusion pattern).

<table>
<thead>
<tr>
<th>( \phi )</th>
<th>It is possible for a state ( \phi ) to occur</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \neg \phi )</td>
<td>is not possible</td>
</tr>
</tbody>
</table>

This pattern represents the concepts of occurrence and its negation, exclusion. It will often be used during the development of
a model to check for the presence or absence of some property that was experimentally observed. For instance, “It is possible for a state with a high concentration of protein P₁ to occur”. Using this pattern, we can also check for mutual exclusion, by using the negation of the pattern in combination with a conjunctive state descriptor. For instance, “It is not possible for a state to occur in which there is a high concentration of protein P₁ and protein P₂”.

More generally, the exclusion pattern captures the safety properties used in the domain of concurrent systems. A safety property (of which mutual exclusion is a typical example) expresses that “something bad never happens” during the execution of the system, in our example a bad state violating mutual exclusion.

**Definition 2 (Consequence pattern).**

If a state \( \phi \) occurs,

then it is necessarily followed by a state \( \psi \)

The consequence pattern relates two events separated in time. More precisely, it expresses that if the first state occurs, then it is possibly or necessarily followed by the occurrence of the second state. If the latter state necessarily follows, then the consequence pattern expresses a form of causal relation. Instances of this pattern are, for instance, “If a state occurs in which protein P is phosphorylated, then it is possibly followed by a state in which the expression of gene g decreases”, or “If a state occurs in which the concentration of protein P is below 5 \( \mu M \), then it is necessarily followed by a state in which the expression of gene g is at its basal level”.

**Definition 3 (Sequence pattern).**

A state \( \psi \) is reached and

is necessarily preceded at some time by a state \( \phi \)

The sequence pattern represents an ordering relation between two events. It ought not to be confused with the consequence pattern, since the conditional occurrence of the second state which characterizes the latter, is absent in the sequence pattern. Both the first and the second state, in that order, have to be observed for an instance of the sequence pattern to be true.

Four variants of the pattern are distinguished, depending on whether the second state follows possibly or necessarily after the first state, and whether the system is in the first state all the time or only at some time before the occurrence of the second state. Instances of this pattern are “A state in which reactions \( R_1 \) and \( R_2 \) occur at a high rate is reached after 2 min, and is possibly preceded at some time by a state in which the transcription factor P is phosphorylated” or “A steady state is reached and is necessarily preceded all the time by a state in which nutrient N is absent”.

**Definition 4 (Invariance pattern).**

A state \( \phi \) can persist indefinitely

The invariance pattern is used to check if the system can or must remain indefinitely in a state. In contrast with the occurrence/exclusion pattern, the question is not whether a particular state can be reached, but rather whether a particular state is invariable. Instances of the pattern are “A state in which reaction \( R \) occurs at a high rate can persist indefinitely” and “A state with a basal expression of gene g must persist indefinitely”.

### 2.4 Translation to temporal logic

By defining a translation into temporal logic of the patterns, the user queries can be automatically cast in a form that allows the verification of the specified property by means of model-checking tools. The patterns defined above are independent of a particular temporal logic, which allows the same high-level specification of a user query to be verified by means of different approaches and tools. It is worth noticing though that some of the patterns we propose have a branching-time nature (e.g., the consequence and the sequence patterns), and therefore these are not translatable into a linear-time formalism, such as LTL (Clarke et al., 1999).

Two examples of translations of the patterns in Sec. 2.3 are shown in tabular form: the Computational Tree Logic (CTL) translation and the \( \mu \)-calculus translation (Table 1). In both CTL and \( \mu \)-calculus, formulas are built upon atomic propositions. Also, the usual connectors of propositional logic, such as negation (\( \neg \)), logical or (\( \lor \)), logical and (\( \land \)) and implication (\( \Rightarrow \)), can be used in both logics. In addition, CTL provides two types of operators: path quantifiers, E and A, and temporal operators, such as F and G. Path quantifiers are used to specify that a property p is satisfied by some (E p) or every (A p) path starting from a given state. Temporal operators are used to specify that, given a state and a path starting from that state, a property p holds for some (F p) or for every (G p) state of the path. Each path quantifier must be paired with a temporal operator. In the case of \( \mu \)-calculus, two types of operators are provided: the least (\( \mu \)) and greatest (\( \nu \)) fixed points, and the modal operators possibility (\( \Diamond \)) and necessity (\( \Box \)). Least and greatest fixed points specify finite and infinite recursive applications of a formula, respectively. For instance, given a state and a path starting from that state, the fact that a property p holds for some state or for all states of the path is expressed using a least (\( \mu \)) or a greatest (\( \nu \)) fixed point, respectively. Modal operators are used to specify that, given a state, a property p possibly (\( \Diamond p \)) or necessarily (\( \Box p \)) holds on the next state of some or all of its outgoing paths.

### 3 CARBON STARVATION RESPONSE IN E. COLI

#### 3.1 Model of carbon starvation response

To test the applicability of the temporal logic patterns, we have used them for the analysis of a model of the carbon starvation response in the bacterium E. coli. In the absence of essential carbon sources in its growth environment, an E. coli population abandons exponential growth and enters a non-growth state called stationary phase. This growth-phase transition is accompanied by numerous physiological changes in the bacteria (Huisman et al., 1996), and controlled by a complex genetic regulatory network integrating various environmental signals.

The molecular basis of the adaptation of the growth of E. coli to the nutritional conditions has been the focus of extensive studies for decades (Gutierrez-Ríos et al., 2007; Hengge-Aronis, 1996). However, notwithstanding the enormous amount of information
accumulated on the genes, proteins, and other molecules known to be involved in the stress adaptation process. It is currently not understood how the response of the cell emerges from the network of molecular interactions. Moreover, with some exceptions (Bettenbrock et al., 2006), numerical values for the kinetic parameters and the molecular concentrations are absent, which makes it difficult to apply traditional methods for the dynamical modeling of genetic regulatory networks.

These circumstances have motivated the development of a qualitative model of the carbon starvation response network using a class of piecewise-linear (PL) differential equations. The PL models, originally introduced by Glass and Kauffman (1973), provide a coarse-grained picture of the dynamics of genetic regulatory networks. They associate a protein concentration variable to each of the genes in the network, and capture the switch-like character of gene regulation by means of step functions that change their value at a threshold concentration of the proteins. The advantage of using PL models is that the qualitative dynamics of the high-dimensional systems are relatively simple to analyze, using inequality constraints on the parameters rather than exact numerical values (Batt et al., 2005, 2008). This makes the PL models a valuable tool for the analysis of the carbon starvation network.

In previous work we developed a PL model that we extend here by the general stress response factor RpoS and related regulators (Ropers et al., 2006; Ropers et al., in preparation). The dynamics of this system are described by nine coupled PL differential equations, and fifty inequality constraints on the parameter values.

### 3.2 Qualitative simulation of carbon starvation response

The mathematical properties of the class of PL models used for modeling the stress response network have been well-studied (Glass and Kauffman, 1973). We have previously shown how discrete abstractions can be used to convert the continuous dynamics of the PL systems into a FSTS (Batt et al., 2008). The states $S$ of the FSTS correspond to hyperrectangular regions in the concentration space, while the transitions $T$ arise from trajectories that enter one region from another. The atomic propositions $AP$ describe, among other things, the concentration bounds of the regions and the trend of the variables inside a region (increasing, decreasing, or steady). The generation of the FSTS from the PL model has been implemented in the computer tool Genetic Network Analyzer (GNA) (Batt et al., 2005). GNA is able to export the FSTS to standard model checkers like NuSMV (Cimatti et al., 2002) and CADP (Garavel et al., 2007).

The application of this approach to the model of the E. coli carbon starvation network generates a huge FSTS. The entire state set consists of approximately $O(10^{10})$ states, while the subset of states that is most relevant for our purpose, i.e., the states that are reachable from an initial state corresponding to a particular growth state of the bacteria, still consists of $O(10^3)$ states. It is clear that FSTSs of this size cannot be analyzed by visual inspection, and that formal verification techniques are needed.

In the next section we show how the patterns defined in Sec. 2.3 can speed up the querying of these FSTSs, by simplifying the formulation of relevant properties to be tested. We are particularly interested in the question how the extension of the model with RpoS affects the predicted dynamics of the system. The instances of the patterns were translated into the temporal logic CTL following the translation rules of Table 1, and then verified using the model-checker NuSMV.

### 4 ANALYSIS OF CARBON STARVATION RESPONSE MODEL USING QUERY PATTERNS

#### 4.1 Mutual inhibition of Fis and CRP

The proteins Fis and CRP mutually inhibit their expression (Fig. 1). The regulatory protein CRP is the target of a signal-transduction pathway, which activates the adenylate cyclase Cya in case of carbon starvation. In turn, the latter synthesizes a small molecule, cAMP, which binds to CRP. This active form of CRP is able to regulate the expression of a large number of genes. In particular,
CRP-cAMP binds to the promoter region of the gene *fis*, thereby preventing synthesis of new Fis proteins. Fis is an important regulator of genes involved in the cellular metabolism but it also inhibits *cpl* expression, by binding to multiple sites in the two promoter regions of the gene, P1 and P2. The regulatory interactions between genes *fs* and *cpl* form a positive feedback loop, a motif often found in the genetic regulatory networks. When present in isolation, this kind of motif has been shown to lead to bistability (Gardner et al., 2000).

The question can be asked whether the motif is also functional in the context of the carbon starvation response network. For instance, the expression of *fs* is not only controlled by CRP-cAMP, but also by the DNA supercoiling level and Fis itself. To check whether the bistability property is preserved in the larger network, we used two instances of the occurrence/exclusion pattern to express that it is impossible that proteins Fis and CRP are simultaneously present at high and at low concentration in the cells at steady state (Table 2). This property was shown to be true by the NuSMV model checker. We conclude that the positive feedback loop involving *fs* and *cpl* is functional.

### 4.2 Damped oscillations after carbon upshift

The carbon starvation response network also contains a negative feedback loop, involving the genes *gyrAB*, *topA*, and *fs* (Fig. 1). GyrAB is a gyrase protein, which supercoils the DNA structure, whereas the topoisomerase TopA relaxes it. An increase of the DNA supercoiling level stimulates expression of Fis, which in turn contributes to controlling the DNA supercoiling level. Hence, the negative feedback loop involving the genes *topA*, *gyrAB*, and *fis* is notably important for the DNA supercoiling level. GyrI represses the activity of GyrAB by forming a complex with the protein. The expression of *gyrI* is notably stimulated by RpoS (Oh et al., 2001). We formulated a consequence pattern to verify whether this affects the functioning of the negative feedback loop. In particular, we checked whether the carbon upshift is still a necessary condition for the occurrence of damped oscillations, as it was in the previous model (Table 2). In the pattern we made use of the state descriptor isOscillatoryState, which labels states as belonging to a (terminal) cycle in the FSTS. The occurrence of an oscillatory state could alternatively be expressed.
true for the query, meaning that the damped oscillations still occur using temporal logic formulas (Sec. 5). The model-checker returned a accumulation of RpoS in the cell. We formulated this question using RpoS to accumulate to a high concentration. RpoS is actively degraded through the protein RssB, which binds to with published data (Qi et al., 1997). Indeed, following carbon starvation, the protein RssB is inactivated, entry into stationary phase, most likely under the influence of RpoS. The second contribution of the paper is the use of these patterns controlled in our conditions: while cells grow on a carbon source, entry into stationary phase cannot occur before RpoS has accumulated. Given the importance of RpoS for the survival of the cell, one may ask whether the entry into stationary phase is always preceded by the accumulation of RpoS in the cell. We formulated this question using a sequence pattern, where the stationary phase is represented by a low level of stable RNAs encoded by the rrn operons (Table 2). The latter indicator is motivated by the fact that stationary-phase cells do not need high levels of these RNAs, which are necessary for the high translational activity of the exponential phase. The property is true, which indicates that the entry into stationary phase cannot occur before RpoS has accumulated.

### 4.3 Control of entry into stationary phase by RpoS

RpoS is a stress sigma factor that allows cells to adapt to and survive under harmful conditions by entering stationary phase (Hengge-Aronis, 1996). Due to its key role, the concentration of RpoS is tightly regulated, at the transcriptional, translational, and post-translational levels. It is mainly the stability of the protein that is controlled in our conditions: while cells grow on a carbon source, RpoS is actively degraded through the protein RssB, which binds to RpoS and targets the factor to an intracellular protease. However, the depletion of the carbon source inactivates RssB, thus allowing RpoS to accumulate to a high concentration.

Given the importance of RpoS for the survival of the cell, one may ask whether the entry into stationary phase is always preceded by the accumulation of RpoS in the cell. We formulated this question using a sequence pattern, where the stationary phase is represented by a low level of stable RNAs encoded by the rrn operons (Table 2). The latter indicator is motivated by the fact that stationary-phase cells do not need high levels of these RNAs, which are necessary for the high translational activity of the exponential phase. The property is true, which indicates that the entry into stationary phase cannot occur before RpoS has accumulated.

#### 4.4 Expression of topA during growth-phase transitions

Our previous model was incapable of accounting for the control of DNA supercoiling during growth-phase transition. In particular, TopA was predicted to be never expressed, which is inconsistent with published data (Qi et al., 1997). The extension of the model with RpoS makes it possible to refine the description of the control of the DNA supercoiling level. On the one hand, GyrAB activity is regulated by GyrI, as mentioned previously, and on the other hand, the topA promoter is activated by RpoS.

In order to know whether topA is expressed in response to the carbon source availability, we used an invariance pattern to check if the absence of topA expression persists indefinitely (Table 2). The corresponding temporal logic formula is false and the diagnostic of the model-checker shows that expression of topA is stimulated at the entry into stationary phase, most likely under the influence of RpoS. Indeed, following carbon starvation, the protein RssB is inactivated, which leads to the accumulation of RpoS at high levels. RpoS in turn stimulates the expression of topA.

## 5 Discussion

Formal verification techniques are promising tools for upscaling the analysis of cellular interaction networks. The widespread adoption of model-checking approaches is restrained, however, by the difficulty for non-expert users to formulate appropriate questions in temporal logics. Inspired by work in the formal verification community (Dwyer et al. (1999), see also Manna and Pnueli (1991)), the first contribution of the paper consists in the formulation of a set of patterns in the form of query templates in structured natural language. The patterns capture a large number of frequently-asked questions by modelers in systems biology, but they are not restricted to a particular type of network, a particular biological system or a particular modeling tool. In addition, we have provided translations of the patterns to two different temporal logics, CTL and mu-calculus. However, the patterns can be translated to other temporal logics provided that they have enough expressive power.

The second contribution of the paper is the use of these patterns for the analysis of the complex genetic regulatory network involved in the carbon starvation response in *E. coli*. We have modeled this using temporal logic formulas (Sec. 5). The model-checker returned true for the query, meaning that the damped oscillations still occur following a carbon upshift.

**Table 2.** Translation of the instances of the patterns used in the analysis of the *E. coli* carbon starvation response into CTL and mu-calculus, following the translation rules in Table 1. The symbol $iSS$ is the abbreviation for the predefined state descriptor $iSS$ steady state.
network by means of PL differential equations and simulated the qualitative dynamics of the system in response to carbon starvation and carbon upshift. Our model extends a previous model (Ropers et al., 2006) with additional global regulators, notably the sigma factor RpoS, to better account for the control of DNA supercoiling during the growth transitions of the bacteria. The patterns are instantiated to verify the effect of this addition to the predicted network dynamics.

The patterns proposed in this paper are globally consistent with those discussed in (Dwyer et al., 1999), but there are differences due to the specific application domain for which our patterns were developed. For instance, the notion of scope used by Dwyer et al. (1999) is not commonly defined for all the patterns, but implicitly present through the use of specific variants for each pattern. Also, we have not explicitly included patterns that can be obtained by the recursive application of other patterns, such as the chain response pattern defined in Dwyer et al. (1999). Present patterns have not been used for the querying of cellular interaction networks thus far, some papers list example questions. It is reassuring to observe that all questions in the list of Chabrier-Rivier et al. (2004) can be expressed by means of the patterns in Sec. 2.3.

An obvious generalization of the patterns proposed in this paper, already briefly mentioned in Sec. 2, would be to allow state descriptors that are formulas in temporal logic. For instance, already briefly mentioned in Sec. 2, would be to allow state descriptors that are formulas in temporal logic. A compromise trading some expressive power for user-friendliness would be to restrict the possible temporal logic formulas to simple forms and introduce these as predefined state descriptors (Sec. 2.3). This is consistent with the main idea underlying the use of patterns, namely that they cannot be expected to cover all possible queries, but rather should allow users to formulate frequent questions without worrying about their translation to temporal logic.

ACKNOWLEDGEMENT

This work was partially supported by FCT program (PhD grant SFRH/BD/32965/2006 to PTM) and PDCT program (project PTDC/EIA/71587/2006). DR, RM, and HDJ were supported by the European Commission under project EC-MOAN (FP6-2005-NEST-PATH-COM/043235).

REFERENCES