Logical and incremental formalization of cell cycle checkpoints

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Checkpoints ensure the integrity of DNA during the cell cycle, which is a succession of molecular and cellular events leading to the division of a mother cell into two genetically identical daughter cells. The DNA is first duplicated (S-phase), then equally distributed between the opposing poles of the cell, which finally divides into two independent daughter cells (M-phase). Two additional phases depict the process of cell preparation before S and M phases: respectively the G1 and G2 phases. Many modeling studies investigating checkpoints are based on ODE systems while the checkpoint concept itself is fundamentally discrete, described as follows: *a checkpoint prevents any event that initiates a phase from taking place before the end of all the events of the previous phase.* So far to our knowledge, very few qualitative models have yet attempted to formally define this discrete concept, as the notion of discrete cell cycle phase is still fuzzy, from a formal point of view.
Therefore, we propose a qualitative modeling study of cell cycle regulation dedicated to the logical specification of the G1, S, G2 and M phases, and the G1/S, S/G2, G2/M and mitosis exit checkpoints. This study has been made possible by using two types of formal methods: the "genetically modified" Hoare logic [1] and the model-checking for CTL [2]. The `TotemBioNet` tool efficiently combines these two methods to exhaustively identify the parameterizations (which govern the dynamics of the regulatory graph) compatible with all formalized biological knowledge [3].
Starting from a qualitative model of cell cycle progression regulation (Behaegel et al. [4]), the cell cycle was defined by a Hoare triple of the form $\{precondition\}\ path\ \{postcondition\}$, where the precondition is a single initial state, the path is a sequence of discrete events, and the postcondition is the single final state. The path was then divided into four canonical phases in order to identify non-permutable key events, which will constitute the main rule of the generic predicate $checkpoint(phase_i, phase_{i+1})$. Since the order of events within a phase is not necessarily known, the predicate (implemented in `Prolog`) includes rules which call `TotemBioNet` to extract all the orders of events of a phase compatible with all other static and dynamic biological knowledge of the system.
The results highlight that three "strong definitions" of checkpoint are validated, but no parameterization satisfies the mitosis exit checkpoint, that requires a *less restrictive* definition. Indeed the single event that can initiate G1 is permutable with the 3 events that can end the M phase. However, no abstract knowledge in our models is challenged since the first event of G1-phase (activation of $CycE/Cdk2$) has also been experimentally observed in M-phase [5]. This new highlighted knowledge has proved that our generic definition of the mitosis exit checkpoint is not biologically consistent, hence has been specifically revised. Finally, the *generic* nature of the checkpoint predicate opens a perspective to study any cyclic phenomenon genetically regulated by checkpoints.

References

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