Exploring the complex interaction patterns of caspases apoptotic signalling pathways in a tuple space-based in silico approach

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Abstract: - In this paper we present a simulation, based on the notion of Biochemical Tuple Spaces for Self-Organizing Coordination (BTS-SOC), for exploring the complex interaction patterns of intracellular signalling networks. The platform is designed to perform in silico experiments, in order to support the research on the design of experiment in vitro. In this platform, the visualization of the concentration values over time and tracking interactions among signalling components allow understanding of intracellular communication processes. In this paper, in particular, we simulated the caspases-signalling pathway; caspases are a family of cysteine proteases, central regulators of apoptosis, cellular self-destruction.

Key-Words: - Apoptotic Signalling Pathways, In Silico Approach, Biochemical Tuple Space, Tuple Space-Based Model

1 Introduction
1.1 Biochemical Tuple Spaces
Biochemical tuple spaces were introduced in [1] as the core of a model for self-organising coordination (BTS-SOC). A biochemical tuple space is a tuple space working as a compartment where biochemical reactions take place. Chemical reactants are represented as tuples, and biochemical laws are represented as coordination laws by the coordination abstraction. Technically, biochemical tuple spaces are built as ReSpecT tuple centres [2], running upon a TuCSoN coordination infrastructure [3]. Tuples are logic-based tuples, while biochemical laws are implemented as ReSpecT specification tuples. In particular, each biochemical tuple space is built around a ReSpecT chemical engine, whose core is an action selection mechanism based on Gillespie algorithm [4] – an algorithm typically used to simulate systems of chemical/biochemical reactions efficiently and accurately and to execute chemical reactions with the proper rate.

1.2 Caspases
Caspases are a family of cysteine proteases (Cysteine dependent, aspartyl specific protease). There are many such caspases within an organism, which work together in a proteolytic cascade, in which they are activated. Cascades are effective means of amplifying a signal to give a stronger response than that one achieved through a single enzymatic reaction. Caspases are synthesized as inactive zymogens (procaspases). At the activation state, caspases undergo two successive proteolysis leading to the appearance of an active heterotetramer formed by the assembly of two large and two small subunits containing two active sites of catalysts [5]. This actively breaks their specific substrates including cytosolic and nuclear proteins. There are two pathways of activation of caspases (Fig. 1) and therefore two pathways for apoptosis: 1) Via receptor, or extrinsic cell death pathway, involving members of the receptor family of Death Receptor factor (DRF) located on the cell surface, and 2) The intrinsic mitochondrial pathway, controlled by members of the family of Bcl-2 proteins. Connection between extrinsic and intrinsic pathways is regulated by Bcl-2 family [6, 7].

1.3 Towards a BTS-SOC-Based In Silico Approach
In this work we show how the BTS-SOC model and infrastructure can be applied to the simulation of complex interaction patterns of caspases apoptotic intracellular signalling pathways. Therefore, initially we present the general BTS-SOC-based model for simulating intracellular signalling systems, along
with a high-level architecture. In the section 3 we develop the modelling and simulation process of the caspases apoptotic signalling pathways, and present the results obtained. Finally, the section 4 is a conclusion.

2 The Complex Interaction Patterns of Caspases Apoptotic Signalling Pathways in a Tuple Space-Based In Silico Approach

In a process of apoptosis, the presence and localization of specific proteins, which activate the signalling caspase pathway, is crucial. In this work we use Biochemical Tuple Spaces for Self-Organizing Coordination, which allows us to properly shape these pathways as described below.

Table 1: Mapping cellular components and structures involved in intracellular signalling onto BTS-SOC abstractions.

<table>
<thead>
<tr>
<th>Cellular components and structures involved in intracellular signalling</th>
<th>Computational abstractions of the BTS-SOC model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular space and intracellular compartments - i.e., extracellular space, membrane, cytosol, nucleus, mitochondria</td>
<td>Tuple centres</td>
</tr>
<tr>
<td>Signalling components - i.e. proteins (membrane receptors, enzymes, regulators, adapters, etc.)</td>
<td>Chemical reactions sets</td>
</tr>
<tr>
<td>Signalling molecules - i.e., ATP, inorganic phosphate, second messengers, etc.</td>
<td>Reactants and concentrations recorded as tuples in the tuple centre</td>
</tr>
</tbody>
</table>

2.1 BTS-SOC-Based Model for Simulation of Intracellular Signalling Pathways

The main components of our BTS-SOC model for simulating intracellular signalling pathways are reported in Table 1, showing how the cellular components and structures involved in intracellular signalling map onto the BTS-SOC computational abstractions. The high-level architecture of the model is depicted in Fig. 2. A detailed explanation of this model can be found at [8].

Fig.1: Simulation of caspases signalling pathways considered for this model.

Fig.2: A high-level architecture for the BTS-SOC-based bioinformatics platform.

It is evident, that the BTS-SOC-based simulation can model the complex caspases-signalling pathway; in the following section we present modelling steps.

3 Modelling and Simulation the Caspases Apoptotic Signalling Pathway

In the methodological workflow in Fig. 3, the major activities to be executed through BTS-SOC-based simulation platform during the modelling and simulation of the caspases apoptotic-signalling pathway are shown.

3.1 Modelling of the Caspases Apoptotic Signalling Pathways

Based on the information reported in the literature, and considering only those elements presented in Figure 1, for both intrinsic and extrinsic pathway,
we proceed to create a table (Table 2), with the following values:

- Identity;
- Concentration in each cellular compartment;
- Free concentration;
- “Bound” concentration;
- Cellular compartment to which it belongs;
- Chemical reactions involving the component and the order in which they occur according to the affinity of the components;
- Reaction temporality situation.

Table 2: Modelling the signalling components belonging to caspases extrinsic apoptotic signalling pathway: an illustrative example. The symbol “@” on the right of an equation indicates the cellular compartment in which the resultant reactant must be registered.

<table>
<thead>
<tr>
<th>Extrinsic pathway</th>
<th>Cellular compartments</th>
<th>Chemical reactions</th>
<th>Km</th>
<th>Vmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular space</td>
<td>DL</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Plasmatic membrane</td>
<td>DL + DR → DR* @ Cytosol</td>
<td>1</td>
<td>10⁻⁵</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DL + DecoryR → DecoryR* @ Cytosol</td>
<td>1</td>
<td>10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>Cytosol</td>
<td>DR* + FADD + Cas8 → Cas8*</td>
<td>4.5</td>
<td>5.8 x 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DR* + FADD + cFLIP + Cas8 → Cas8</td>
<td>2.3</td>
<td>5.8 x 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cas8* + ProCas3 → Cas3*</td>
<td>50</td>
<td>5 x 10⁻⁵</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cas8* + ProCas6 → Cas6*</td>
<td>33.7</td>
<td>3 x 10⁻⁵</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cas8* + ProCas7 → Cas7*</td>
<td>20</td>
<td>1 x 10⁻⁵</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cas9* + ProCas3 → Cas3*</td>
<td>100</td>
<td>5 x 10⁻⁴</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Simulation of the Caspases Apoptotic Signalling Pathways

As it can be seen from Fig. 3, the simulation of an intracellular signalling pathway in BTS-SOC-based bioinformatics platform involves the following three major phases:

1) Creating cellular compartments. A tuple centre (BTS) is required for each cellular compartment involved in the signalling pathway to be simulated. In our study, four tuple centres (plasmatic membrane, cytosol, mitochondrial membrane and mitochondria) are required to model four intracellular compartments (see Fig. 5).
2) Introducing reactants. In order to set up the simulation system, reactants should be introduced in the BTS. First of all, each reactant belongs to a specific cellular compartment—so, it has to be put in the appropriate BTS. Initially, only the pre-existing reactants — *i.e.*, those reactants already in the compartments before the signalling pathway is activated — have to be put in the BTS (see Fig. 6).

3) Setting chemical reactions. The last step in setting up the simulation is the introduction of the reactions modelling the behaviour of signalling pathway. In our model, based on the Gillespie algorithm, every chemical reaction has a rate that expresses (along with the concentration of the input elements) the probability of the transformation (see Fig. 7).

Fig.5: Five cellular compartments: Extracellular space, Plasmatic membrane, Cytosol, Mitochondrial membrane and Mitochondria - required for the simulation of caspases signalling pathway - have been created.

Fig.6: Introduction of reactants in the *cytosol* BTS.

Fig.7: Setting chemical reactions.

### 3.3 In Silico Experiment Results

Our modelling and simulation methodology initially considers the intrinsic and extrinsic pathways for apoptosis as shown in Fig. 1. The extrinsic pathway of apoptosis begins with the death signals (hormones, growth factors, cytokines, stress, etc.); these signals trigger two types of response through extrinsic and intrinsic pathways. The modelling and simulation of these events are represented in Table 1 and Fig. 5, 6 and 7. Take just one example for the simulation, as shown in Fig. 1. The effectors caspases 3, 6, 7 are activated as a consequence of the activation of extrinsic or intrinsic pathway. Caspase-3 is critical for apoptosis and it is activated in the cytoplasm, however, two hours after being activated it can be located at the plasma membrane in the cytoplasm and nucleus. Figures 8 to 17 show the simulation results of these events in the BTS-SOC-based bioinformatics infrastructure.

Fig.8: Concentration-time curves: from the activation of Death Receptor (DR) to activation of Caspase-8 (Cas8) in the extrinsic apoptotic pathway.
Fig.9: Concentration-time table: from the activation of Death Receptor (DR) to activation of Caspase-8 (Cas8*) in the extrinsic apoptotic pathway.

Fig.10: Concentration-time curves: from the activation of Caspase-8 (Cas8) to Apoptosis (Apop) in the extrinsic apoptotic pathway.

Fig.11: Concentration-time table: from the activation of Caspase-8 (Cas8) to Apoptosis (Apop) in the extrinsic apoptotic pathway.

Fig.12: Concentration-time curves: inhibition of the extrinsic apoptotic pathway by protein family core inhibiting apoptosis (IAPs) and protein inhibitor of caspase-8 (FLIP).

Fig.13: Concentration-time table: inhibition of the extrinsic apoptotic pathway by protein family core inhibiting apoptosis (IAPs) and protein inhibitor of caspase-8 (FLIP).

Fig.14: Concentration-time curves: mitochondrial or intrinsic pathway.
4 Conclusion

When running the simulation, we observe on the molecular level how cancer cells evade caspases signalling pathways. This platform is very useful because it allows one hand to design experiments and on the other to determine protein-protein interactions invisible. A prioritized action plan can then be put together all pathways: PKC, MAPK/ERK and PI3K/AKT, toward true integration of apoptosis.

References: