Predictive Modelling of Stem Cell Differentiation and Apoptosis in *C. elegans*

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Abstract. The nematode *Caenorhabditis elegans* has been established as a modeling organism in biomedical research for several decades. Its hermaphrodite germ line encompasses key developmental concepts like stem cell differentiation and apoptosis; therefore it provides a good model system for these basic concepts. Here, we have extended and refined our previous computational model, which encompasses developmental landmarks and the resulting movement of germ cells along the gonadal tube. We have used the molecular dynamics (MD) framework to model the physical movement of cells due to the force arising from cell divisions. The model simulation was calibrated with experimental time and it is in accordance with experimental observations. In addition, the model provides means for testing hypotheses regarding the behaviour of mutated germ lines and the potential mechanisms causing physiological apoptosis, which are difficult to assess experimentally.

Keywords: Computational modelling, apoptosis, *C. elegans*, differentiation, molecular dynamics, germ line.

1 Introduction

Since the 1970s [1], the nematode *Caenorhabditis elegans* has been used as a modelling organism in biomedical research. In particular, its hermaphrodite germ line, shown in Fig. 1 A, provides a good model for basic developmental concepts like stem cell differentiation and apoptosis. The specific organisation of the germ line (cf. Fig. 1 B) with distinct developmental zones corresponding to specific ranges along the longitudinal axis of the germ line makes it an ideal candidate for formal analysis. Here, we have extended and refined our previous computational model [2], which encompasses these developmental landmarks and the resulting movement of germ cells

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along the gonadal tube. We have used the molecular dynamics (MD) framework to model the physical movement of cells due to the force arising from cell divisions. The model simulation was calibrated with experimental time and it is in accordance with experimental observations. In addition, the model provides means for testing hypotheses regarding the behaviour of mutated germ lines and the potential mechanisms causing physiological apoptosis, which are difficult to assess experimentally.

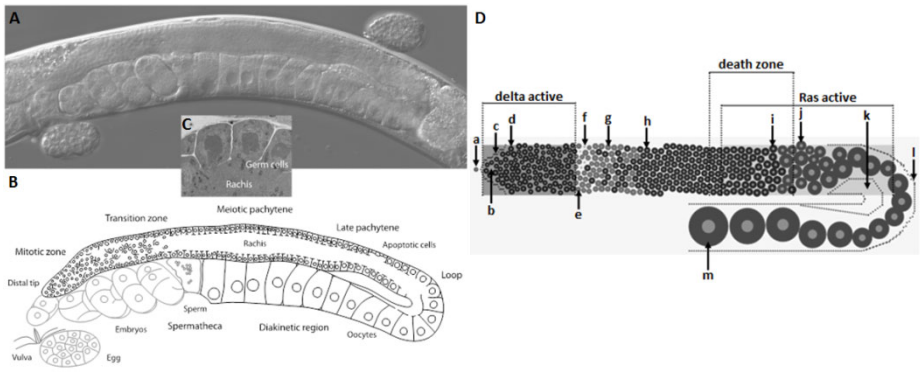

Fig. 1. The germ line of *Caenorhabditis elegans*. A: Differential interference contrast (DIC) microscopy image of the germ line shown along a plane through the centre of the gonad tube in a live animal. The head of the worm is to the right, the posterior gonad to the left of the picture. The germ cells in the meiotic pachytene region form a monolayer around a concentric inner tube (rachis), seen as a nuclei-free area in this picture. B: Schematic picture of A with developmental zones indicated. C: Cross section of the germ line by transmission electron microscopy shows germ cell monolayer with shared cytoplasm (rachis). D: Snapshot of an execution of the germ line model with zones of ligand activity and death zone indicated; a: Distal Tip Cell, b: marked cell , c: border of germ line, d: mitotic cell with highest Notch level, e: mitotic cell with Notch level between highest and 0.5 times the highest level, f: mitotic cell with Notch level between 0.5 times the highest level and 0, g: cell with GLD-1 level higher than Notch level, h: meiotic cell, i: meiotic cell that has grown to about twice its original size, j: oocyte with Ras level above threshold, k: rachis, l: border of germ line, m: fully grown oocyte.

The organisation of the germ line into distinct developmental zones is due to different signalling pathways. In the first part of the germ line, delta ligand causes the Notch level within the germ cells to peak and keeps them competent to divide. This area is superseded by a zone of GLD-1 activity whose relative levels inside the cells decide about entry into meiosis. Germ cells that have entered meiosis start to differentiate into oocytes and accumulate Ras in a specific area of the germ line with external Ras stimuli. If a cell reaches a certain level of Ras, it becomes an oocyte. The oocytes grow until they fill the diameter of the tube at the end and exit into the spermatheca to become fertilised. Corresponding to the location of the first oocytes, just before the loop in the wild-type germ line, there is a defined death area. Apoptosis is confined to this specific zone and does not happen anywhere else in the germ line.

2 Model Construction

Here, we present a dynamic computational model of the germ line of *C. elegans* based on the molecular dynamics framework. The model encompasses the previously mentioned developmental features and extends a previous model [2]. The basic component of our model is the MD movement algorithm that results in the movement of cells as they react to pressure from surrounding cells that divide, grow or are pushed themselves. That is, the major driving forces of movement in the model are cell growth and division. In this updated version of the model, we account for the special architecture of the germ line (cf. Fig. 1 A to C) with germ cells lining the outside of the tube and the shared cytoplasm in the middle of the germ line. In our two-dimensional model this three-dimensional aspect is realised using periodic boundary conditions that allow cells to interact with each other at the top and the bottom of the tube and to "jump" from top to bottom and vice versa (cf. Fig. 1 D). Furthermore, we have included the mitosis/meiosis decision due to Notch and GLD-1 in the model as follows. When a cell is in the zone of GLD-1 activity, its Notch level decays and its GLD-1 level accumulates. The cell stays in mitosis as long as the GLD-1 level is less than the Notch level. As soon as this changes, the cell enters meiosis but finishes growing until it has the size of a cell just before division (cf. Fig. 1 D). For better tractability, we changed the cell division to depend on a Gaussian distribution with average 20,000 steps and standard deviation 4,000 steps divided by 3. If we set an hour to be equivalent to 1,000 steps this assures an average time of 20 hours between divisions and a general range from 16 hours to 24 hours, which is according to the literature [3,4]. We have implemented the occurrence of apoptosis in a random, timed fashion for calibration purposes. That is, every time a cell dies the model assigns a new random time of death between 0 and 1,000 steps (~ 1h) from the current time at which another random cell within the death zone dies.

3 Results

We have calibrated our model according to literature values [3-6] and our own laboratory observations. For example, we have simulated constitutive Notch gain of function with our model, which resulted in a tumourous germ line filled with mitotic cells and no differentiating cells, which matches the experimental results [7]. This gives us confidence in the validity of the model and its appropriateness for testing further hypotheses.

The living adult germ line is difficult to assess experimentally especially since the animal moves while observing it and it is difficult to track molecular factors on a single cell level. We have used this model to perform *in silico* experiments hoping to shed new light on behaviour of mutant germ lines and on the mechanisms causing developmental apoptosis. The results of these could be used to direct experimental research. In order to evaluate the viability of these experiments we have tested a Ras loss of function mutation and the effects of increased division rate. The model predicts that a Ras loss of function mutation in the whole germ line causes a failure in

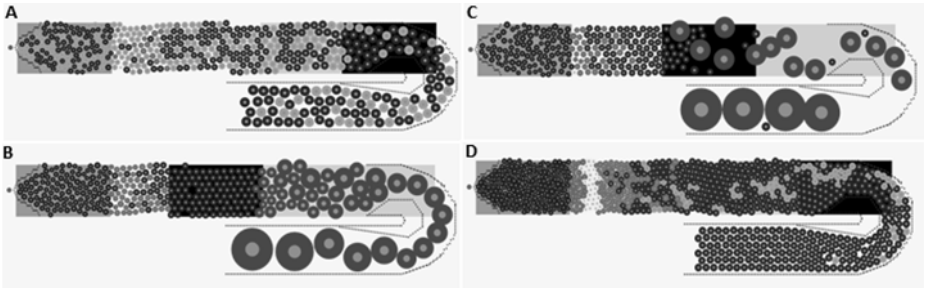

Fig. 2. Snapshots of executions of the germ line model with different mutant backgrounds. A: Ras loss of function mutation prevents differentiation into oocytes and keeps cells in meiotic pachytene. B: Ras gain of function results in death zone and oocytes to be located more distally. C: Lower division rate results in very distal oocytes and cells being more spread out. D: Higher division rate causes germ line to fill with mitotic cells and build a tumour.

A

	Division	Movement	Death	Fertilisation	Variation	Look
Reference	20h	90min	2/h	2/h		
Random death	993=1h	110min	2.04/h	2.14/h	*timed*	good
density > 0.95	998=1h	101min	2.07/h	2.06/h	med.	med.
density, no eggs			too high			
Ras < thres.			too high			
lowest Ras	1002=1h	117min	1.97/h	2.34/h	*timed*	med.
highest Ras	995=1h	106min	1.98/h	2.2/h	*timed*	med.
size > 1.329123	992=1h	107min	1.99/h	2.25/h	good	good
smallest size	1001=1h	118min	2/h	2.23/h	*timed*	med.
biggest size	997=1h	117min	2/h	2.2/h	*timed*	med.
time in zone > 16000	1002=1h	94min	2.18/h	1.93/h	bad	med.
dev. time < thres.			too high			
dev. time > 67500	991=1h	100min	2.01/h	2.11/h	med.	med.
dev. time > 64500, no eggs	993=1h	110min	2/h	2.29/h	med.	good

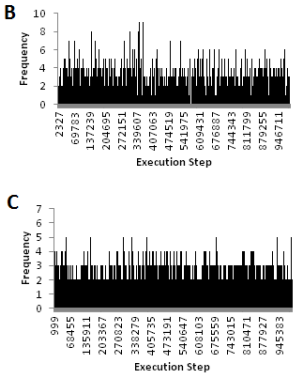

Fig. 3. In *silico* experiments on possible apoptosis mechanisms. A: Table showing the values extracted from the tested hypotheses and the reference values for division, movement, death and fertilisation rates as well as personal evaluations of variation in death and fertilisation rates and the general look of the execution. Hypotheses shaded dark gray were not evaluable, hypotheses in light gray performed best in all respects. B: Death rate per hour variation over the whole execution of cell death if size > 1.329123. C: Fertilisation rate per hour variation over the whole execution of cell death if size > 1.329123.

differentiation. The germ line is filled with meiotic cells that do not differentiate into oocytes, as shown in Fig. 2 A. A gain of function in Ras, on the other hand, causes oocytes to be present more distally in the germ line of our model than under normal conditions (cf. Fig. 2 B). Furthermore, the death zone is located more distally. The model predicts that a division rate lower than normal results in very distal oocytes and a more spread out distribution of cells, as illustrated in Fig. 2 C. For an increase in the division rate, the model shows a tumourous germ line filled with mitotic cells

(cf. Fig. 2 D). These results have not been tested experimentally yet, but they are in accordance with our expectations.

We use the model to direct our study of the mechanisms causing apoptosis. We have tested *in silico* six possible causes for apoptosis: completely random death, high density, Ras levels, size, time spent in death zone and developmental time (i.e. time since last division). The outcomes of these simulations are shown in Fig. 3 A. For lowest and highest Ras as well as smallest and biggest size, we used a timed death in the fashion of random death, where cells die at certain random steps of the execution, as previously explained. Fig. 3 A shows that, surprisingly, these timed deaths did not perform as well as the random death. Also, apoptosis caused by a high density did not perform as well as we had expected. In fact, the mechanisms performing best are random death and apoptosis caused by large cell size or a high developmental time (in both cases eggs are defined incompetent to die). Indeed, Fig. 3 B and C show that the death and fertilisation rates per hour for apoptosis caused by a large cell size don't show a lot of variation. The prediction of our model that large size is a very likely cause for apoptosis in the germ line makes a lot of sense biologically, assuming that the apoptotic germ cells work as nursing cells for the oocytes and, by dying, donate cytoplasm for their benefit. In this scenario, it seems effective to deplete a large cell rather than a small one. Since cells probably do not 'measure' their size, it can be considered as a phenotype of the "real" cause. Looking at our results, this could very well be the developmental time. Accordingly, we intend to focus our experimental efforts in the direction highlighted by the model. However, as previously stated, this could be very hard. We find that the computational model provides valuable predictions and inspiration for new avenues to explore experimentally *in vivo*.

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