THREE DIMENSIONAL MATHEMATICAL MODELLING OF PRONUCLEI MIGRATION FOR THE MOUSE

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ABSTRACT

It is still an open question when the orientation of the embryonic-abembryonic axis of the mouse embryo is laid down. The two most explicit symmetry breaking events for the egg are the extrusion of the second polar body and the sperm entry. The main question addressed in this paper is what happens between the sperm entering the egg and fusion of the two pronuclei. Orientation of the apposing pronuclei probably plays a decisive role in the polarity of the developing embryo. In order to shed some lights on this intriguing question, a mathematical model that describes the pronuclei dynamics have been constructed in the form of a stochastic differential equation. The model concerns pronuclei migration from the time of the sperm entry to the fusion and spatial orientation of this fusion. The methodology consists of using stacks of confocal microscopy time-lapse images of the pronuclei migration together with statistical methods to identify realistic parameters in the model. Given different angles between the sperm entry and the position of the second polar body, the final model is then used to produce distributions of orientations of the meeting positions between the pronuclei. However, the main result is the suggested model itself which describes the main features of the migration. The fitted model is based on two forces of attraction. Migration is directed towards the centre but also towards the other pronucleus. Parameter values corresponding to the size of these forces are estimated from data of both eggs treated with a microtubule inhibitor and untreated eggs. Simulations from the model with the different model parameters are accomplished and distributions of meeting positions are plotted. These simulated distributions could for instance be used as initial value distributions for future models of egg cleavage.

Keywords: Confocal Microscopy, Developmental Biology, Image Analysis, Mathematical Modelling, Migration, Pronucleus.

INTRODUCTION

Developmental biology is the biology of the first events the living species experience. As the microscopical techniques improves and the amount of data increases the need for better and more effective analysis methods is developing. In this area there is a great interest towards a higher usage of methods from biophysics and biomathematics. Mathematical modelling of the microtubule dynamics in the *C. elegans* egg are used in for instance (Kimura and Onami, 2005), (Dogterom *et al.*, 2005) and many others.

The work of this paper has its background in the research of the Magdalena Zernicka-Goetz group at the Gurdon Institute in Cambridge. The group studies development of spatial patterning and determination of cell fate of the early mouse embryo.

As most mammalian eggs, the mouse egg is a highly regulative system. In being so, it is still an open question of when cell fates are fixed, (Davies and Gardner, 2002), (Davies and Gardner, 2003), (Gardner, 2003), (Hiiragi and Solter, 2004), (Motosugi *et al.*, 2005), (Plusa *et al.*, 2005), (Zernicka-Goetz, 2005), (Zernicka-Goetz, 2006). There exists different viewpoints of definitions of total randomness and pattern bias against rigidity and determinative behaviour. However, our aim is to show that these two possible explanations do not exclude each other but can both simultaneously affect the cell fates. Cells may have a pattern, or a set of rules, to follow and at the same time be influenced of the stochasticity of the chemical system and the cytoplasmic environment inside the egg.

At this time it remains unanswered how the embryonic-abembryonic axis of the mouse blastocyst is first established. Cell-fate is flexible meaning that the development can recover from perturbations (Gilbert, 2006). Previous results indicate that the first cleavage is preferably occurring along the short axis of the egg (Zernicka-Goetz, 2005). The short axis is in turn related to sperm entry and migration of the pronuclei. The second polar body and the sperm entry point could together make up a coordinate system for the axis formation of the later embryo. Other studies of

the mouse development show deviating results of when patterning is initiated in the egg. For instance (Davies and Gardner, 2002) and (Hiiragi and Solter, 2004). Some of these studies that conclude that the pattern formation starts later in the embryo have however been conducted in 2D. However, the authors of this paper think it is important to consider this as a 3D problem since ignoring one of the three dimensions may introduce some bias.

One purpose of having a model for the migration is to be able to more easily visualize the fertilization process to answer these questions. A model of this kind could be used to predict outcomes from for instance the point of sperm entry by simulating different scenarios. It could provide initial conditions to further models of cell division and differentiation. Moreover, values of model parameters can be used to quantify treatment or measurement effects on the egg.

We are using inspiration from developmental biology literature and potential theory to produce a model for the migration. In setting up the model, data from confocal time-lapse DIC images of mouse eggs have been used to record the 3D-coordinates of the pronuclei up until the first division of the egg.

MATERIALS AND METHODS

Confocal microscopy DIC time-lapse images of mouse eggs where used as data input for the migration model. The imaging method is described more thoroughly in Plusa *et al.* (2005). Recordings of the pronuclei positions have been carried out, for all three dimensions. The measuring procedure is performed, until the first division, positions are expressed as (m_x, m_y, m_z) for male pronucleus and (f_x, f_y, f_z) respectively for female. Some of the time points have errors in them, e.g. missing slices or inconclusive images, so that they must be discarded. Hence the time points used are not always evenly spaced.

Each sequential image consists of seven z-scans with 14 µm distance apart. A mouse egg has a diameter of about $80 - 100$ µm. The width and height of each pixel correspond to 0.37 µm. This gives an uncertainty which is quite large in coordinates perpendicular to the image planes (z-axis) compared to the image plane coordinates (x- and y-axes). Sequential images are taken with 5 minutes intervals. Figure 1 is an example of a single time-lapse image at the third z-scan level of one egg. Both the female and the male pronuclei are visible as fairly circular structures in the egg.

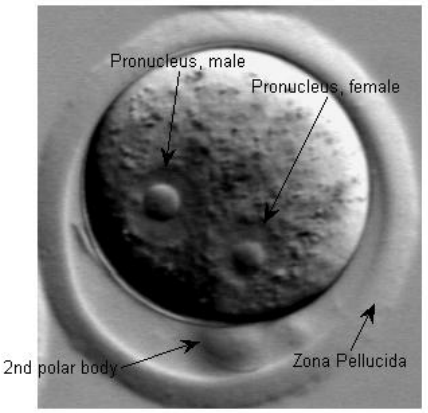

Fig. 1. *An example of a time-lapse image of a mouse egg. The pronuclei are visible, the female pronucleus to the right and male to the left in the image respectively.*

It is the artificial coordinate system introduced by the imaging technique of confocal microscopy that is used for the analysis. The first two coordinates are set by the image plane and the third is set by the z-stack. One purpose of having this non-natural coordinate system is to keep control over the low resolution in the z-axis. Strange values or results for this coordinate could then be related back to this property. At this stage of the modelling the egg is assumed to be spatially homogeneous for simplicity. How this relates to the actual environment of the pronuclei, may be questioned. Cytoplasm can be denser in some directions if the egg is flattened. For instance, during sperm entry the egg is believed to have an oval rather than a circular shape (Zernicka-Goetz, 2005). Also the cytoplasm and the cytoskeleton undergo quite large changes during the fertilization. This processes could lead to lesser or larger crowding in the surrounding cytoskeleton of the pronuclei. In this paper, modelling is conducted on a macroscopic level, meaning that we will address the pronuclei mechanics and not the cytoplasm directly. Hence no molecular reactions have been modelled either. The assumptions of homogeneity leads to the same kind of behaviour in all directions of the egg.

Manual observations of the migration indicate that not only the pronuclei move towards the centre of the egg but also toward each other. For example, the experiments by Hamaguchi and Hiramoto (2008) show this kind of behaviour of sand dollar eggs. Therefore in our model below the pronuclear migration is assumed to depend on two forces of attraction, F_C and F_A. This could be seen as simple representations of a more complex force compound. Where F_C represents attraction towards the centre and F_A represents

attraction towards the other pronucleus. See also Figure 2. Note that the size of the force F_A is equal of strength for both pronuclei but oppositely directed.

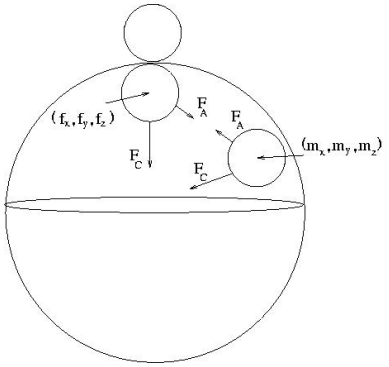

Fig. 2. *Schematic picture of the two attraction forces. The positions of the pronuclei are recorded with three coordinates regarding the position in the image plane (*x- *and* y*-coordinates) and estimated height (*z*-coordinate) in the z-stack.*

When the male and the female pronucleus move toward the centre and to each other, the migration depends on microtubule guiding the movements. The microtubule are bipolar structures which are involved in many cellular processes such as mitosis and cytokinesis, (Gilbert, 2006), (Wolpert *et al.*, 1998). They are guided from the centrosomes provided by the sperm and the female pronucleus. Along the microtubule, motor proteins can move towards both the plus end and the minus end of the microtubule (kinein and dynein). The intracellular medium is a very crowded environment and pronuclei migration is basically movement at low Reynolds numbers, see for instance (Purcell, 1977) and (Forgacs and Newman, 2005). A world of this kind leads to a model with only frictional forces and no inertia. Frictional forces are mainly due to the surrounding cytoplasm and cytoskeleton. If only frictional forces are present, the forces should be proportional to the velocity of the pronucleus. The total force compound acting on a pronucleus will then be $F_A + F_C$.

First we give a model for F_C, which represents the centring part of the forces acting on the pronucleus. The special structure of the microtubule will give rise to both pulling and pushing forces. It might be difficult to see which one to choose in a simple model like ours. Very few, if any, results can be found for mammalian species. Kimura and Onami (2005) however write about the length-dependent pulling force as primary mechanism for the *C. elegans* male pronuclear migration. This primary mechanism has been modelled such as having longer microtubule leads to a more powerful force. Microtubules that grow towards the cortex of the egg have a limited length, which leads to that the microtubules directed along the centre of the egg are the longest and also give a stronger pulling force. The pronuclei would migrate towards the centre. The idea is to have a force that is based on friction and does not blow up at the centre or at the boundary of the egg, when pronuclei are moving against the drag from the cytoplasm. Note that the modelling is conducted in 3D, and $\mathbf{m}$ and $\mathbf{f}$ are 3D locations of the male and female pronucleus respectively. We make the following ansatz for the centring part

$$\frac{d\mathbf{f}}{dt} = C\frac{-\mathbf{f}}{(R-|\mathbf{f}|+\varepsilon)|\mathbf{f}|}, \qquad (1)$$

for the female pronucleus, and analogously for the male pronucleus. Here R is the radius of the egg and C, ε constants that have to be estimated. The model in (1) gives a velocity for the pronucleus which flattens out at the centre of the egg. Close to the centre this force will be smaller and close to the boundary of the egg it will be larger. The parameter ε assures that the velocity is not too large right at the boundary of the egg. Equation (1) builds on the assumption that the pronuclei move with the same speed to the centre of the egg and hence have the same constant C.

Next, we model F_A, the attraction of the pronuclei toward each other. This force is modelled by finding inspiration in the theory of electrostatics and charged particles, which is reasonable given the bipolarity of the microtubule. It is desirable that the attraction increases with decreasing distance between the pronuclei. We make the following addition to the earlier ansatz of Equation (1)

$$\frac{d\mathbf{f}}{dt} = C\frac{-\mathbf{f}}{(R-|\mathbf{f}|+\varepsilon)|\mathbf{f}|} + A\frac{\mathbf{m}-\mathbf{f}}{(|\mathbf{m}-\mathbf{f}|^{\alpha+1}+\gamma)}, \qquad (2)$$

for the female pronucleus. The additional term to the differential equation for the male dynamics is almost equivalent but added with opposite sign. The constant γ is added for gaining a more feasible model for the pronuclei dynamics. We will here give a motivation for Equation (2) with $\alpha = 2$. In (Payne *et al.*, 2003) it is suggested that higher density of microtubule connected to the other pronuclei yields a higher force. Given that the distance between the approaching pronuclei is ρ, and the radius of a pronuclei is r, we get from similarity that $\frac{r}{h} \approx \frac{\rho}{r}$, see Figure 3. Here h is the height of the intersecting microtubule area of one pronucleus. This area is then proportional to the quantity $\frac{r^4}{\rho^2}$, hence the resulting force is proportional to one over the squared distance between the pronuclei. The force of attraction is modelled to depend on how big the connecting "microtubule surface" is. If more

microtubules are connected it should yield a larger force. This is the first basic assumption we make.

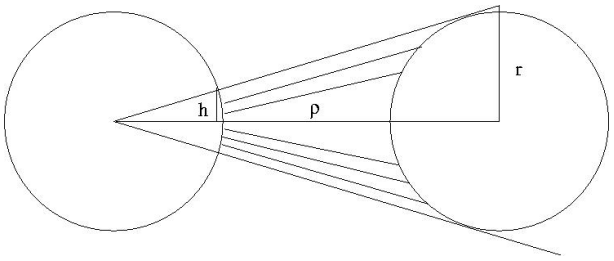

Fig. 3. *Illustration of how the attraction between pronuclei is modelled. The attractional force is modelled to depend on the connected area of microtubules sent out. Similarity yields a force proportional to* $1/\rho^2$*, where rho is the distance between the pronuclei.*

The environment in the mouse egg is not simple fluid material. It is on the contrary quite complex and the microtubule guided migration is not fulfilled without resistance from the surrounding cytoplasm. This leads to a natural variation in the data which needs to be taken into account for into the model.This random variation is modelled as Brownian motion as

$$d\mathbf{B}(t) = \mathbf{B}(t+dt) - \mathbf{B}(t) = \\ = (B_x(t+dt) - B_x(t), B_y(t+dt) - B_y(t), \\ B_z(t+dt) - B_z(t)), \qquad (3)$$

where $B.(t) \sim N(0,\sigma^2)$. Here we have assumed that the egg is spatially homogenous. This makes it easier to estimate the variation of the noise since then the variation is assumed to be equal for all axes. The noise is added to Equation (2) yielding the following complete stochastic differential equation model for the dynamics of the female pronucleus

$$\frac{d\mathbf{f}}{dt} = C\frac{-\mathbf{f}}{(R-|\mathbf{f}|+\varepsilon)|\mathbf{f}|} + A\frac{\mathbf{m}-\mathbf{f}}{(|\mathbf{m}-\mathbf{f}|^{\alpha+1}+\gamma)} + d\mathbf{B}(t), \qquad (4)$$

RESULTS

The model parameters C, A, ε, γ and α are estimated by a nonlinear least squares method. It is the Line-Search type algorithm that is used in the optimization. The best fit is given by $\varepsilon = 0$, $\gamma = 0$, $C = 0.05R$, $A = 0.09R$ and $\alpha = 1.81$. Where, R is the radius of the egg. Not that the value of α is not far from the assumed value of 2. The standard deviation of the noise is estimated to $\sigma = 0.02R$. It should be noted that despite the limited data the values of the parameters are quite reasonable. Parameter estimation leads to the following final version of the model.

$$\frac{d\mathbf{m}}{dt} = C\frac{-\mathbf{m}}{(R-|\mathbf{m}|)|\mathbf{m}|} + A\frac{\mathbf{f}-\mathbf{m}}{|\mathbf{m}-\mathbf{f}|^3} + d\mathbf{B}(t), \qquad (5)$$

and analogously for the female pronucleus.

In order to visualize the consequences of the model for the migration and to see how well the model describes the data, simulations have been performed and the simulated pronuclei dynamics have been plotted. For one specific egg the starting coordinates have been used in one run of the simulation and the result is shown in Figure 4. The actual trajectories recorded are plotted on Figure 5. It can be seen that at least this simulated trajectory looks like the one in the real data. The model succeeds to mimic some of the behaviour that is observed. However there seems to be a larger spread in the simulated data than in the real ones. This may be a feature of the analyzed eggs having quite large individual variation.

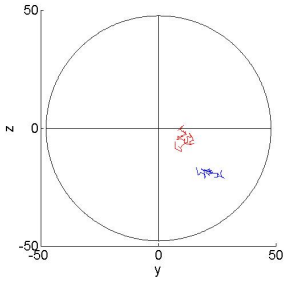

Fig. 4. *Plot of simulated data from one run of the model in the yz-plane. The male trajectory is plotted with blue colour and the female with red respectively.*

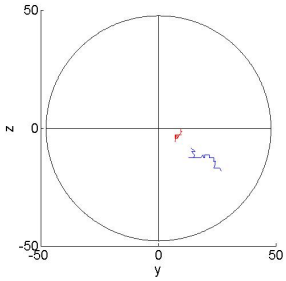

Fig. 5. *Plot of manual data for egg 31 in the yz-plane. The male trajectory corresponds to blue colour and the female to red respectively.*

Monte Carlo simulations of the dynamics are carried out ten thousand times to produce a distribution of trajectories for the pronuclei. At each simulation the meeting position is recorded, or rather the angles that specify the vector between the centre points of the

approaching pronuclei. Simulations of this kind could in turn provide initial conditions for future models regarding cleavage of the egg. It could be interesting to see what kind of distributions of meeting positions different sperm entry points would lead to.

The two dimensional distribution for both the azimuthal (θ, the angle around the equator of the egg) and elevational (ϕ) angle of the vector between the pronuclei is recorded. See Figure 6, where this have been plotted for a sperm entry of 45° from the second polar body. The polar body is always at the north pole and the sperm entry point (SEP) is varied in the elevational angle but held at azimuthal angle 0°. Meeting planes are concentrated in the upper hemisphere of the egg, hence positive latitudinal angles. Note that azimuthal angle of 180°, is equal to azimuthal angle of −180°. Red colour means high counts and blue lower.

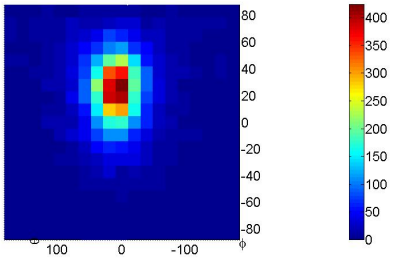

Fig. 6. *Simulation of pronuclei meetings, yielding a distribution of the azimuthal and elevational angle of the vector between the pronuclei centers. Sperm entry is at an elevational angle of* 45°.

According to this model a sperm entry further from the second polar body would introduce a wider spread of where the pronuclei meet and possibly also a wider spread of the cleavage plane. The main weight in the angular distributions seems to be positioned at the same place for the different sperm entry points.

We also have data available from two eggs treated with Cytochalasin B (CB). CB is known for being an inhibitor of microtubule growth. This substance is sometimes used for slowing down the migration to be able to gain images of improved quality. We have examined what the consequences of the CB treatment are for the model parameters. What is more important than the actual results for our model is the possibility of using parameter estimates from data of diverging characteristics in simulations. Results from these estimations could in turn be used for testing if techniques or treatments used are too invasive.

The parameters for the pronuclear migration are reestimated for the CB data with the same method as before. Again ε and γ are estimated as zeros. The centring constant $C = 0$ and the attraction constant $A = 0.02R$. Furthermore $\alpha = 1.99$ and $\sigma = 0.005R$, and R is, as before, the radius of the egg. Since CB hinders the microtubule to grow, the rate of migration would be expected to decrease. This can be seen in the values of the parameter estimates as lower force constants. The centring force seems actually to have vanished.

Simulations of meeting positions have also been done with the parameter values estimated from the CB data. The results are shown in Figure 7. At a first glance, the distributions in Figures 6 and 7 seem similar. However, looking closely it can be seen that the spread is lower in the distribution for the eggs treated with CB. According to the simulations we get that a lower centring force gives a smaller spread in the positions where pronuclei meet. If these positions are important in the future cleavage of the egg this may be an indication of an undesired invasiveness of the treatment.

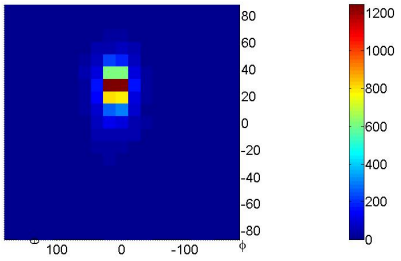

Fig. 7. *Simulated distributions of meeting angles for the data treated with Cytochalasin B. Compare with Figure 6. Since the centering constant is set to zero a lower spread of the distribution is achieved.*

DISCUSSION

We have introduced a model for the pronuclei migration in the mouse egg and described how it can be applied to actual data of fertilized mouse eggs. The model is a first attempt to describe such processes at a mechanical level. The model is based on a few simple assumptions and is meant to serve as the starting point for a more complex and biologically more relevant model. The model was even reduced further given the data, to Equation (5). In spite of being simple, the model still gives some nice results and an idea of what could be done and tested. Our results indicate that the CB treatment affects the pronuclei migration, which is something we would like to investigate further.

However there are a lot of issues to address before going further. As we have already mentioned, the data available is quite limited. In order to increase the accuracy of the estimation, one would need a

variety of eggs with pronuclei starting in uniformly distributed distances from each other. As the imaging technique continuously improves it is possible to have a better resolution in both the image plane data and a smaller distance between the z-scan levels. Fertilization dynamics differ quite largely from egg to egg but z-scan resolution is still the biggest limitation of the data. However, one should not push this too far. It is important not to use imaging techniques that affect the migration in a not desired way as the egg is a sensitive system.

Our main aim is to obtain a model that is more consistent with the developmental biological literature than our current model is. It may be preferable to start with modelling the cytoskeleton in a more thorough way, since this is what the microtubule actually clings to when they are engaged in the pronuclear migration. Our intention is to make use of better analysis methods such as semiautomatic or automatic image analysis methods for tracking the pronuclei in the image sequences. It is desired to remove as many human errors as possible. Image analysis methods are not only useful for increasing objectivity but also for making it possible to analyze pronuclei trajectories more effectively. The manual measuring procedure is not an easy task but something that should be performed by an experienced person with good knowledge of the migration process.

Finally, it should be recognized that the model gives expected results for especially the eggs treated with the microtubule inhibitor. Also, we give a motivation for having a certain expression for the attraction between the pronuclei which is in turn specified by estimating the parameters from the data.

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