Influence of the Nuclear Membrane, Active Transport, and Cell Shape on the Hes1 and p53–Mdm2 Pathways: Insights from Spatio-temporal Modelling

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Abstract There are many intracellular signalling pathways where the spatial distribution of the molecular species cannot be neglected. These pathways often contain negative feedback loops and can exhibit oscillatory dynamics in space and time. Two such pathways are those involving Hes1 and p53–Mdm2, both of which are implicated in cancer.

In this paper we further develop the partial differential equation (PDE) models of Sturrock et al. (J. Theor. Biol., 273:15–31, 2011) which were used to study these dynamics. We extend these PDE models by including a nuclear membrane and active transport, assuming that proteins are convected in the cytoplasm towards the nucleus in order to model transport along microtubules. We also account for Mdm2 inhibition of p53 transcriptional activity.

Through numerical simulations we find ranges of values for the model parameters such that sustained oscillatory dynamics occur, consistent with available experimental measurements. We also find that our model extensions act to broaden the parameter ranges that yield oscillations. Hence oscillatory behaviour is made more robust by the inclusion of both the nuclear membrane and active transport. In order to bridge the gap between in vivo and in silico experiments, we investigate more realistic cell

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geometries by using an imported image of a real cell as our computational domain. For the extended p53–Mdm2 model, we consider the effect of microtubule-disrupting drugs and proteasome inhibitor drugs, obtaining results that are in agreement with experimental studies.

**Keywords** p53 · Hes1 · Nuclear membrane · Active transport · Cell shape

## 1 Introduction

Intracellular signal transduction is an inherently spatial process. The eukaryotic cell hosts a variety of spatial compartments (e.g., nucleus, cytoplasm). Each compartment permits different metabolic activity and is often separated from the rest of the cell by a thin lipid membrane. The appropriate subcellular localisation of molecular species is critical for a cell to remain healthy (Kim et al. 2000; Johansson et al. 2008; Norvell et al. 2005). Signalling molecules reach the appropriate spatial compartments through molecular movement, such as diffusion and active transport, and these mechanisms of movement are functions of location and time. Cell shape and size change over time and are known to influence intracellular signal transduction (Meyers et al. 2006; Neves et al. 2008). The key process of transcription occurs at specific sites, genes, in the nucleus, and some genes are located closer to the nuclear membrane than others, increasing their sensitivity to transcription factors (Cole and Scarcelli 2006). Nuclear pore complexes determine where species move in and out of the nucleus and how quickly they do so (Cangiani and Natalini 2010). Within the cytoplasm, another key process, translation, occurs in the ribosomes. It is apparent from all these observations that mathematical models of intracellular signalling pathways will be more realistic the more they seek to account for spatial features inherent to these pathways.

Many intracellular pathways exhibit oscillatory dynamics in space and time in response to a range of external stimuli (Hirata et al. 2002; Geva-Zatorsky et al. 2006; Nelson et al. 2004; Shankaran et al. 2009). A negative feedback loop often lies at the core of such pathways, controlling the levels of mRNA and proteins which are frequently transcription factors. These molecules initiate or regulate transcription in eukaryotic cells, and in order for them to function, they must bind to specific DNA sequences in the nucleus. Two examples of negative feedback loops are the Hes1 pathway (which plays a role in somitogenesis) and the p53–Mdm2 pathway (which is critical for regulating the cell-cycle). Both can be deregulated in human cancer (Sang et al. 2008; Lane 1992). When quantitative data are available, mathematical models can help delineate these highly complex pathways and even make predictions that experimentalists can check (Locke et al. 2005).

In comparison to temporal models, there are few spatio-temporal models of intracellular signalling pathways in the literature, although the body of work is growing. Early attempts at spatio-temporal modelling of intracellular pathways containing negative feedback loops were carried out by Glass and co-workers, who recognised the inherent spatial heterogeneity of cells and observed oscillatory dynamics for activator-inhibitor kinetics (Glass and Kauffman 1972; Shymko and Glass 1974). Mahaffy et al. subsequently developed models to capture spatial features for such
pathways, introducing delays for transcription and translation and oscillatory dynamics were again observed (Mahaffy and Pao 1984; Busenberg and Mahaffy 1985; Mahaffy 1988). More recently, Gordon et al. developed a partial differential equation (PDE) model for the p53–Mdm2 pathway (Gordon et al. 2009) including delays and which produced sustained oscillations. It was solved in two spatial dimensions, but did not consider separate compartments for the nucleus and cytoplasm. Other PDE models not containing delays have also appeared recently including that of Terry et al., who studied the Notch and NF-κB pathways, finding oscillatory behaviour that closely resembles experimental results (Terry et al. 2011; Terry and Chaplain 2011).

Spatio-temporal models of intracellular processes have been investigated not only in the context of negative feedback loops. For instance, Kholodenko and co-workers have considered general reaction-diffusion models of protein kinase and phosphatase activity within cells (Brown and Kholodenko 1999; Kholodenko 2006), Cangiani and Natalini have examined active transport of proteins along microtubules (Cangiani and Natalini 2010), and Dinh et al. have studied intracellular trafficking of adenoviral vectors (Dinh et al. 2005). For a review of modelling intracellular spatio-temporal interactions, see Rangamani and Iyengar (2007) and Kholodenko (2006). The development of models which reflect spatial and temporal aspects of intracellular pathways can be regarded as a first step towards an effective computational approach in investigating conditions under which pathways become deregulated and in the optimising of targeted drug treatment.

Mathematical models of the Hes1 and p53–Mdm2 pathways have been developed by Sturrock et al. (2011). Building on previous modelling approaches, PDEs were used to capture the spatio-temporal evolution of the pathways. For a particular set of parameter values, the systems produced sustained oscillations, spatially and temporally, accurately reflecting experimental evidence. Through computational simulations, ranges of values for diffusion rates, degradation rates, and Hill coefficients were found such that sustained oscillations occurred. Furthermore, by mathematically varying the location of the ribosomes, an “optimal” range of distances outside the nucleus for protein synthesis was presented.

In this paper, we consider several extensions of these PDE models for Hes1 and p53–Mdm2, with the main aims being to examine the roles of the nuclear membrane, active transport, and cell shape. First, we explore different boundary conditions at the nuclear membrane. It is clear from experimental evidence that accurate mathematical models of intracellular signalling pathways should account for activity at the nuclear membrane. For example, transport of tumour suppressors and oncogene products across the nuclear membrane has been reported to be disrupted in cancer cells (Kim et al. 2000). It has also been suggested that modifying nuclear-cytoplasmic transport activity may block tumorigenesis (Kau et al. 2004). Therefore, it is important to model the nuclear membrane in detail, which we shall do by taking into account its thickness and the rate at which mRNA and protein molecules diffuse across it. Second, we consider active transport of protein molecules from the cytoplasm to the nucleus. Some molecules, especially transcription factors, must overcome high cytoplasmic viscosity and travel significant distances in small time intervals. Movement by diffusion alone may not be enough to overcome high cytoplasmic viscosity. However, rapid
translocation becomes possible when motor proteins bind to transcription factors and then actively transport them along microtubules. The inclusion of active transport is achieved mathematically in our models through the introduction of convection terms. Finally, we study how cell shape affects the Hes1 and p53–Mdm2 pathways. In order to investigate more realistic cell geometries, we import an image of a real cell. We note that it is not possible to consider such extensions in models comprised solely of ordinary differential equations (ODEs). Using PDEs allows spatial effects to be examined explicitly and facilitates the study of how protein localisation is regulated.

The format of this paper is as follows. In Sect. 2, we describe the Hes1 PDE model from Sturrock et al. (2011) and extend the simulation study of this model, finding ranges of values for all model parameters such that sustained oscillatory dynamics occur. We then develop the model further by including a nuclear membrane and active transport. For both Hes1 protein and hes1 mRNA, plots of total nuclear and cytoplasmic concentration over time and spatial profiles are presented and discussed. The effects of microtubule-disrupting drugs and of varying the cell shape are considered. In Sect. 3, we begin by describing a slightly modified version of the p53–Mdm2 PDE model from Sturrock et al. (2011) and explore this model numerically on a domain that is an imported image of a real cell, finding ranges of values for model parameters which yield sustained oscillatory dynamics. We then extend this model to include a nuclear membrane and active transport. Total nuclear and cytoplasmic concentration plots for all model species (namely, p53, p53 mRNA, Mdm2, Mdm2 mRNA), and spatial profiles for p53 and Mdm2, are presented. We investigate the effects of microtubule-disrupting drugs and proteasome inhibitor drugs on the extended p53–Mdm2 model, obtaining results in agreement with experimental studies. We summarise our work and suggest ways to extend it in Sect. 4. A few technical details and additional plots are deferred to an Appendix. Finally, animations of the numerical solution of the extended p53–Mdm2 model that are in close agreement with experimental movie clips are included as supporting information files.

## 2 The Hes1 Pathway

Hes1 is a member of the family of basic helix-loop-helix (bHLH) transcription factors. It has been found to be deregulated in some forms of cancer (Sang et al. 2008) and is known to play a role in somitogenesis, the developmental process responsible for segmentation of the vertebrate embryo. During somitogenesis, a “segmentation clock” controls the timing of the assignment of mesodermal cells to discrete blocks. The segmentation clock depends on the oscillatory expression of a complex network of signalling pathways, including the Hes1 pathway which contains a negative feedback loop (see Fig. 1). This feedback loop is formed through interactions of the Hes1 protein with its own gene, where the Hes1 protein binds to regulatory sequences on the hes1 promoter and represses the transcription of hes1 mRNA. Experiments have measured the concentration levels of hes1 mRNA and Hes1 protein in mouse cell lines (Hirata et al. 2002; Masamizu et al. 2006). In response to serum treatment, it was found that levels of
Hes1 mRNA and Hes1 protein exhibited oscillations with a regular period of approximately two hours. This coincides with the period observed for the mouse segmentation clock.

Mathematical models of oscillatory dynamics in the Hes1 pathway have taken a variety of forms. The first attempt to model this pathway was presented in Hirata et al. (2002), where an ODE model was used. However, in order to reproduce the observed oscillations, a third unknown species was introduced. Monk (2003) found that invoking an unknown species could be avoided by introducing a delay term (representing the processes of transcription and translation), producing a delay differential equation (DDE) system. The effect of low particle numbers in this system was explored in Barrio et al. (2006). The details of the Hes1 pathway were scrutinised in greater depth in Momiji and Monk (2008), again using a DDE system. In particular, an investigation into the effects of dimerisation and compartmentalisation was presented. The role of Gro/TLE1 was considered in Bernard et al. (2006). Other models have examined the role of the Hes1 pathway in somitogenesis (Agrawal et al. 2009), while Sturrock et al. (2011) explored spatio-temporal oscillations in a PDE model.

2.1 Hes1 Mathematical Model

We begin by introducing the PDE model developed in Sturrock et al. (2011) describing the intracellular interactions between hes1 mRNA (concentration denoted by $[m]$) and the Hes1 protein (concentration denoted by $[p]$). The model is considered on a two-dimensional spatial domain representing a cell, with a separate nucleus and cytoplasm. In the equations below, a subscript $n$ denotes a nuclear concentration, and a subscript $c$ denotes a cytoplasmic concentration.

We assume that both protein and mRNA are subject to diffusion. Diffusion coefficients are denoted depending on the type of species (either a subscript $m$ for mRNA or $p$ for protein) and location (a subsubscript $n$ for nuclear or $c$ for cytoplasmic). For example, the diffusion coefficient for hes1 mRNA in the nucleus is $D_{mn}$. Both protein and mRNA are assumed to undergo linear decay, with parameter $\mu_m$ denoting mRNA decay and $\mu_p$ denoting protein decay. Production of mRNA takes place by the process of transcription in the nucleus. Our production term for nuclear hes1 mRNA is a Hill-like function which decreases as protein levels in the nucleus increase. In this function, the parameters $\alpha_m$, $\hat{p}$, and $h$ represent, respectively, the basal rate of transcription in the absence of nuclear protein, the concentration of Hes1 protein that reduces the transcription rate to half its basal value, and a Hill coefficient.
Proteins are translated from mRNA by ribosomes in the cytoplasm, a process that is likely to occur at least some minimal distance from the nuclear membrane (see the appendix of Sturrock et al. 2011). Hence we assume that protein production occurs a small distance outside the nucleus with production rate proportional to the amount of cytoplasmic hes1 mRNA, the constant of proportionality being denoted $\alpha_p$. The full system of equations is therefore given by:

$$\frac{\partial [m_n]}{\partial t} = D_{m_n} \nabla^2 [m_n] + \frac{\alpha_m}{1 + ([p_n] / \hat{p})^\delta} - \mu_m [m_n], \quad (1)$$

$$\frac{\partial [m_c]}{\partial t} = D_{m_c} \nabla^2 [m_c] - \mu_m [m_c], \quad (2)$$

$$\frac{\partial [p_c]}{\partial t} = D_{p_c} \nabla^2 [p_c] + H_1(x, y) \alpha_p [m_c] - \mu_p [p_c], \quad (3)$$

$$\frac{\partial [p_n]}{\partial t} = D_{p_n} \nabla^2 [p_n] - \mu_p [p_n], \quad (4)$$

where $H_1(x, y)$ is a function accounting for the localisation of protein production in the ribosomes a distance $l$ from the centre of the nucleus and is defined as follows:

$$H_1(x, y) = \begin{cases} 0 & \text{if } x^2 + y^2 \leq l^2, \\ 1 & \text{if } x^2 + y^2 > l^2. \end{cases} \quad (5)$$

We apply continuity of flux boundary conditions across the (internal) nuclear membrane and zero-flux boundary conditions at the outer cell membrane:

$$D_{m_n} \frac{\partial [m_n]}{\partial n} = D_{m_c} \frac{\partial [m_c]}{\partial n}, \quad \text{and} \quad [m_n] = [m_c] \quad \text{at the nuclear membrane}, \quad (6)$$

$$D_{p_n} \frac{\partial [p_n]}{\partial n} = D_{p_c} \frac{\partial [p_c]}{\partial n}, \quad \text{and} \quad [p_n] = [p_c] \quad \text{at the nuclear membrane}, \quad (7)$$

$$\frac{\partial [m_c]}{\partial n} = 0 \quad \text{at the cell membrane}, \quad (8)$$

$$\frac{\partial [p_c]}{\partial n} = 0 \quad \text{at the cell membrane}, \quad (9)$$

where $n$ is a unit normal. We also apply zero-initial conditions, i.e.,

$$[m_n] = [m_c] = [p_n] = [p_c] = 0 \quad \text{at } t = 0. \quad (10)$$

The PDE system given by (1)–(4) subject to conditions (6)–(10) was explored numerically in Sturrock et al. (2011), where parameter values were found such that oscillatory dynamics occur. However, it was by no means an exhaustive exploration, and we have extended it here. To solve the Hes1 system numerically, we used the same technique as in Sturrock et al. (2011). First we non-dimensionalised the model, details of which are provided in the Appendix. Then we chose non-dimensional parameter values which were obtained from Eq. (25) in Sturrock et al. (2011). We solved
the model numerically using the finite element method as implemented in the software package COMSOL 3.5a, using triangular basis elements and Lagrange quadratic basis functions along with a backward Euler time-stepping method of integration. This numerical method of solution is used in all the simulations in this paper. The equations were solved on the domain shown in Fig. 2, representing a cell with cytoplasmic and nuclear subdomains. Typical simulations took from 55 seconds (Hes1 system) to 1200 seconds (p53–Mdm2 system). Finally we calculated dimensional parameter values—these are shown in the third column in Table 1. The calculations are described in the Appendix. For simplicity, all nuclear and cytoplasmic diffusion coefficients were set equal to the same constant; we denote the dimensional diffusion coefficient by $D_{ij}$, which indicates diffusion of species $i$ (mRNA or protein) in location $j$ (nucleus or cytoplasm).

For our new simulation study, we have found ranges of values for all of the parameters such that the system exhibits sustained oscillatory dynamics, where we define such dynamics as being able to observe at least five distinct peaks in the total concentration of the transcription factor in the nucleus. These ranges are given in the fourth column in Table 1. To find the range for any particular parameter, we varied this parameter whilst holding all the other parameters fixed at their “default” values, the dimensional versions of which are stated in the third column of Table 1. For simplicity, we investigated only integer Hill coefficients. The meaning and use of non-integer Hill coefficients is discussed in Zeiser et al. (2007) and Prinz (2010).

### 2.1.1 Hes1 Model Parameter Values

Our range for the diffusion coefficient in Table 1 is consistent with two recent spatio-temporal modelling studies of intracellular signalling pathways similar in scope to the present study (Terry et al. 2011; Terry and Chaplain 2011). Experimentalists have found the diffusion coefficient of soluble proteins in the cytoplasm to be in the range $10^{-9}$ to $10^{-8}$ cm$^2$ s$^{-1}$ (Matsuda et al. 2008; Seksek et al. 1997), which is in agreement with the upper bound of our range. There is also a growing body of evidence...
suggesting that proteins and mRNA molecules are subject to macromolecular crowding, which generates an environment where diffusion is hindered by obstacles and traps (Mendez et al. 2010). Taking this into account would likely increase our lower bound to be consistent with experimental measurements. We may consider macromolecular crowding in future work.

The degradation rate $\mu_m$ for $hes1$ mRNA and the degradation rate $\mu_p$ for Hes1 protein have both been estimated from experiments. Hirata et al. estimated $\mu_m$ to be $4.83 \times 10^{-4} \text{ s}^{-1}$ and $\mu_p$ to be $5.16 \times 10^{-4} \text{ s}^{-1}$ (Hirata et al. 2002). Our parameter ranges for $\mu_m$ and $\mu_p$ in Table 1 contain these experimental estimates.

We mentioned above in Sect. 2 that a DDE model of the Hes1 pathway had been explored in Monk (2003). Our range for the Hill coefficient, namely $h \geq 4$, is very similar to the range ($h > 4$) producing sustained oscillatory dynamics in the DDE model in Monk (2003). Note that a larger Hill coefficient corresponds to greater non-linearity, or co-operativity, in the regulation of $hes1$ transcription by Hes1 protein. According to Monk (2003), Hes1 acts as a dimer, which alone would suggest that $h = 2$, so the requirement that $h > 2$ implies that there may be interactions between the three binding sites for Hes1 at the hes1 promoter.

Our value for the critical concentration of Hes1, namely $\hat{p} = 10^{-9} \text{ M}$, is the same as the critical concentration for the zebrafish Her1 protein mentioned in Lewis (2003). Her1 is similar to Hes1 in that both are believed to belong to simple negative feedback loops. The DDE model of the Hes1 pathway in Monk (2003) contains a parameter (namely, $p_0$) analogous to $\hat{p}$ but representing a number of molecules rather than a concentration. A sensible range is suggested as 10 to 100. By an elementary calculation converting concentration into number of molecules, we find that our value for $\hat{p}$ corresponds to approximately 16 molecules, which clearly lies within the postulated range in Monk (2003).
Our estimate for the translation rate $\alpha_p$ of 0.0555 s\(^{-1}\) is similar to the rate mentioned in Bernard et al. (2006) of 1 min\(^{-1}\) or 0.0167 s\(^{-1}\). The basal transcription rate $\alpha_p$ has not been measured experimentally, so we leave our estimate of $6.25 \times 10^{-11}$ M s\(^{-1}\) as a prediction. The distance $l$ of translation from the centre of the nucleus has been studied in Figs. 9–11 in Sturrock et al. (2011), but a range of values such that sustained oscillatory dynamics occur was not stated. The range for $l$ presented in Table 1 reveals that oscillatory dynamics can be obtained when the minimum distance of protein translation coincides with where the cytoplasm meets the nucleus. However, increasing $l$ too much results in a loss of oscillatory dynamics, implying that the precise spatial location of the ribosome in the cytoplasm is important.

2.2 Extended Hes1 Model

In this section we extend the original Hes1 model of Sturrock et al. (2011) (described in Sect. 2.1), first by including explicitly the structure of the nuclear membrane. Encapsulating the nucleus, the nuclear membrane divides the cell into two compartments, between which there is a constant exchange of molecular material. This physical separation of the nucleus and cytoplasm provides a level of spatial regulation in signal transduction. Nucleocytoplasmic transport occurs through the nuclear pore complex (NPC). The NPCs perforate the two lipid bilayers which form the nuclear membrane and allow for bidirectional transport of a large number of RNA and protein cargoes which vary in size from 1 kDa to nearly 50 MDa (almost 40 nm in diameter) (Weis 2003). The number of functional NPCs varies depending on the growth state of the cell, which in turn affects the overall permeability of the nuclear membrane (Feldherr and Akin 1991).

In order to model the nuclear membrane explicitly, we need to account for its thickness $d$ (which is also the depth of the NPC) and the diffusion of molecules across it. This effectively allows us to model its permeability. The nuclear membrane thickness has been estimated to be approximately 100 nm (Beck et al. 2004). Regarding diffusion across the nuclear membrane, note that since the NPCs are not located everywhere within it, there exist some areas of it that cannot be traversed, and this slows down the average rate at which particles diffuse across it. Molecular crowding may also slow down this average rate. In the restricted space of an NPC, larger molecules, such as proteins, will diffuse more slowly than smaller molecules, such as mRNA (Marfori et al. 2011; Rodriguez et al. 2004). A second explicit step in modelling the nuclear membrane is therefore to assume that diffusion across it is slower than in the cytoplasm or nucleus, with protein diffusion slower than mRNA diffusion across the membrane. Although diffusion coefficients for cytoplasmic, nuclear, and nuclear-embedded proteins have been estimated experimentally (Klonis et al. 2002), we are not aware of experimental estimates for diffusion rates across the NPCs for hes1 mRNA and Hes1 protein. Therefore, still assuming as at the end of Sect. 2.1 that the nuclear and cytoplasmic diffusion coefficients are the same constant $D_{ij}$, we shall simply choose $D_m = D_{ij}/5$ and $D_p = D_{ij}/15$ for the nuclear membrane diffusion coefficients for hes1 mRNA and Hes1 protein, respectively. In summary, we can take into account nuclear membrane thickness and slower diffusion across it by
replacing the boundary conditions in (6) and (7) by those for a thin boundary layer of width \(d\), defined by:

\[
\begin{align*}
D_{m_n} \frac{\partial [m_n]}{\partial n} &= \frac{D_m ([m_n] - [m_c])}{d}, \\
D_{m_c} \frac{\partial [m_c]}{\partial n} &= \frac{D_m ([m_c] - [m_n])}{d}, \\
D_{p_c} \frac{\partial [p_c]}{\partial n} &= \frac{D_p ([p_c] - [p_n])}{d}, \\
D_{p_n} \frac{\partial [p_n]}{\partial n} &= \frac{D_p ([p_n] - [p_c])}{d}.
\end{align*}
\]

The boundary conditions (11)–(14) describe the flux across the nuclear membrane. This flux can be thought of as a permeability coefficient (defined as the diffusion coefficient of the species in the nuclear membrane divided by the membrane thickness) multiplied by the concentration difference of the species across the nucleocytoplasmic boundary.

Our second extension to the original Hes1 model of Sturrock et al. (2011) is to consider active transport. As we mentioned in the Introduction, it is important for transcription factors to be able to move quickly from the cytoplasm to the nucleus, which can be achieved by active transport along microtubules. The microtubules are fibrous, hollow rods that function primarily to help support and shape the cell. For the majority of the cell cycle (i.e., the interphase period), the microtubules are arranged in the cytoplasm as an aster originating from the microtubule-organising centre (MTOC) located close to the nucleus (see Fig. 1; Lomakin and Nadezhdina 2010). The microtubules also play a major role in the intracellular trafficking of macromolecules and organelles (Cole and Lippincott-Schwartz 1995; Cangiani and Natalini 2010). This trafficking of cargo molecules occurs as follows: motor proteins bind to the cargoes and then actively transport them along microtubules. Motor proteins can be split into two families, dyneins (which move molecules from the cytoplasm towards the nuclear membrane) and kinesins (which move molecules towards the cell membrane). Motor proteins interact with microtubules via their ATPase domain, while their opposite terminus interacts with the cargo being transported. The movement of proteins along microtubules towards the nucleus can be viewed as a biased random walk. For example, although cargoes bound to dynein mainly move in the direction of the nucleus, there is evidence for detachment and reattachment of cargoes to motor proteins, pauses, and simultaneous attachment to both dynein and kinesin which can change the direction of movement through a “tug-of-war” (Muller et al. 2008; Smith and Simmons 2001). For simplicity, we shall model active transport of the transcription factor Hes1 as always being directed towards the nucleus. We do this by adding a convection term to the cytoplasmic Hes1 equation, namely (3), which becomes

\[
\frac{\partial [p_c]}{\partial t} = D_{p_c} \nabla^2 [p_c] - \nabla \cdot (\mathbf{a} [p_c]) + \alpha_p [m_c] - \mu_p [p_c],
\]

active transport
Fig. 3  Plot showing the vector field \( \mathbf{a} \) (defined in (16)) modelling the convective effect of the microtubules.

Fig. 4  Diagram showing the domain used in numerical simulations of the extended Hes1 model. Spatial units here are non-dimensional, with one non-dimensional spatial unit corresponding to 10 µm. The cell is an ellipse, centre (0, 0), with major and minor axes of 3 and 2, respectively. The nucleus is shown as a blue circle, centre (0, 0), radius 0.3. The microtubule-organising centre (MTOC) is located around the circumference of the circle, centre (0, 0), radius 0.35, which surrounds the nucleus and is close to it. The cytoplasm is the part of the cell that is outside the nucleus (the green and orange regions), and active transport occurs only in the green region. It does not occur in the orange region because microtubules originate from the MTOC and not from the nucleus.

where \( \mathbf{a} \) is the convective velocity given by

\[
\mathbf{a} = \left[ \frac{-ax}{\sqrt{x^2 + y^2}}, \frac{-ay}{\sqrt{x^2 + y^2}} \right],
\]

and the parameter \( a \) is the convection speed. The vector field \( \mathbf{a} \) is depicted in Fig. 3.

Finally, in order to take into account the location of the MTOC, we modify the domain on which our equations are solved. To be specific, we solve on the domain shown in Fig. 4. In this domain, the MTOC is located around the circumference of a circle a small distance away from the nucleus. Since microtubules originate from the MTOC and not from the nucleus, we assume that active transport may occur only in the green region outside the MTOC. Hence, in the outer green region, we assume that cytoplasmic Hes1 protein satisfies (15), but in the orange region between the MTOC and the nuclear membrane we assume that it satisfies (3).
2.2.1 Simulation Results for Extended Hes1 Model

We solved the extended Hes1 model numerically (given by (1)–(4) and (15)), subject to conditions (8)–(14), on the domain shown in Fig. 4. We retained the parameter values used to simulate the original Hes1 model in Sect. 2.1. The diffusion coefficients across the nuclear membrane have already been defined in terms of the diffusion coefficient in the nucleus and cytoplasm, so did not need to be estimated. The nuclear membrane thickness was chosen to be the same as the experimentally measured value of 100 nm (Beck et al. 2004). The rate of active transport was chosen to produce numerically stable sustained oscillations. We summarise the parameter values used for the extended Hes1 model in the second column of Table 2. Ranges of values such that the model exhibits sustained oscillatory dynamics were also found and are stated in the third column of Table 2.

By comparing Tables 1 and 2, we see that the parameter ranges yielding sustained oscillatory dynamics are widened by the addition of an explicit nuclear membrane and active transport. Our extended model is therefore both more realistic and a more robust oscillator. Note in particular that sustained oscillations may occur in the extended model even when the Hill coefficient $h$ is as low as two. As we mentioned above in Sect. 2.1.1, Hes1 acts as a dimer, which suggests that oscillations should be possible with $h = 2$ (Monk 2003). We have now found that this is indeed possible, and so it may not be necessary to seek evidence for binding site interactions or other nonlinearities to faithfully model the Hes1 pathway. Furthermore, by fixing $h = 2$ we studied parameter sensitivity and found ranges of the nuclear membrane parameters (i.e. permeability) and active transport speed which produce oscillatory dynamics. As can be seen in Table 3, these ranges are quite broad and suggest that allowing for a Hill coefficient of 2 could be a generic feature of systems including a nuclear membrane and active transport. Oscillatory dynamics are observed over a wider range of the parameter $l$. This is due to the fact that active transport moves proteins created close to the cell membrane towards the nucleus, ensuring enough protein accumulates in the nucleus to shut down hes1 mRNA production.

It has been estimated that motor proteins transport cargo along microtubules at a speed of approximately $5.00 \times 10^{-5}$ cm s$^{-1}$ (Smith and Simmons 2001). Our value for the rate of active transport in the second column of Table 2, namely $a = 1.25 \times 10^{-6}$ cm s$^{-1}$, is lower than this estimate, but it should be kept in mind that our value incorporates not only transport but also implicitly incorporates reactions required for such transport, such as binding to and dissociation from microtubules, as well as competition between newly synthesised molecules of Hes1 protein to attach to microtubules. It should also be kept in mind that molecules can become temporarily stuck on microtubules, slowing down the average rate of active transport (Smith and Simmons 2001). A more advanced study of active transport would require consideration of stochastic effects, in which context it might be fruitful to apply the Gillespie algorithm (Barik et al. 2010; Kar et al. 2009; Barik et al. 2008). In any case, our range of values for the active transport rate such that sustained oscillatory dynamics occur does include the estimate of $5.00 \times 10^{-5}$ cm s$^{-1}$. Moreover, our range of values for the nuclear membrane thickness indicates that the numerical solution is robust to variation in this parameter, which is reassuring as this value is likely to vary between cells.
Figure 5a shows how the total nuclear concentrations of hes1 mRNA and Hes1 protein vary over time, and Fig. 5b shows how the total cytoplasmic concentrations of hes1 mRNA and Hes1 protein vary over time. By comparing Figs. 5a and 5b with, respectively, Figs. 4 and 5 in Sturrock et al. (2011), we see that oscillatory dynamics are retained in the model when a nuclear membrane and active transport are added to it. Yet there are some quantitative differences between our new plots and those for the original model. For example, a greater proportion of Hes1 enters the nucleus in our new plots, for whereas in Sturrock et al. (2011) the heights of the peaks in nuclear Hes1 were only approximately 2.5% of those in the cytoplasm, Fig. 5 shows that they are now approximately 33% of those in the cytoplasm. Thus, although our new assumption of slow diffusion across the nuclear membrane hinders the entry of Hes1 into the nucleus, our other new assumption of cytoplasmic active transport of Hes1 is more than enough to overcome this. The increased proportion of Hes1 protein in the nucleus influences the production of hes1 mRNA. To be specific, since Hes1 is a transcription factor which inhibits its own gene, then hes1 mRNA production is reduced by the increased proportion of nuclear Hes1 protein. In particular, hes1 mRNA levels in the nucleus drop to zero between consecutive peaks in Fig. 5a, a result not encountered in Fig. 4 in Sturrock et al. (2011).

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value in simulations</th>
<th>Range over which oscillations are observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{ij}$</td>
<td>$3.13 \times 10^{-11} \text{ cm}^2 \text{s}^{-1}$</td>
<td>$6.67 \times 10^{-12} \text{ cm}^2 \text{s}^{-1}$ to $1.13 \times 10^{-9} \text{ cm}^2 \text{s}^{-1}$</td>
</tr>
<tr>
<td>$\alpha_m$</td>
<td>$6.25 \times 10^{-11} \text{ M} \text{s}^{-1}$</td>
<td>$\geq 2.50 \times 10^{-12} \text{ M} \text{s}^{-1}$</td>
</tr>
<tr>
<td>$\hat{p}$</td>
<td>$1.00 \times 10^{-9} \text{ M}$</td>
<td>$3.17 \times 10^{-11} \text{ M}$ to $7.69 \times 10^{-7} \text{ M}$</td>
</tr>
<tr>
<td>$h$</td>
<td>5</td>
<td>$\geq 2$</td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>$1.25 \times 10^{-3} \text{ s}^{-1}$</td>
<td>$2.08 \times 10^{-4} \text{ s}^{-1}$ to $4.00 \times 10^{-3} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$\alpha_p$</td>
<td>$0.0555 \text{ s}^{-1}$</td>
<td>$\geq 2.50 \times 10^{-3} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$\mu_p$</td>
<td>$1.25 \times 10^{-3} \text{ s}^{-1}$</td>
<td>$2.08 \times 10^{-4} \text{ s}^{-1}$ to $3.79 \times 10^{-3} \text{ s}^{-1}$</td>
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<tr>
<td>$D_m$</td>
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<td>$\geq 2.50 \times 10^{-14} \text{ cm}^2 \text{s}^{-1}$</td>
</tr>
<tr>
<td>$D_p$</td>
<td>$2.08 \times 10^{-12} \text{ cm}^2 \text{s}^{-1}$</td>
<td>$\geq 1.67 \times 10^{-14} \text{ cm}^2 \text{s}^{-1}$</td>
</tr>
<tr>
<td>$d$</td>
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</tr>
<tr>
<td>$a$</td>
<td>$1.25 \times 10^{-6} \text{ cm} \text{s}^{-1}$</td>
<td>$7.50 \times 10^{-9} \text{ cm} \text{s}^{-1}$ to $1.08 \times 10^{-4} \text{ cm} \text{s}^{-1}$</td>
</tr>
<tr>
<td>$l$</td>
<td>6.32 $\mu$m</td>
<td>Nuclear membrane (3 $\mu$m) to 10.7 $\mu$m</td>
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</table>

Table 3

<table>
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<tr>
<th>Parameter</th>
<th>Value in simulations</th>
<th>Range over which oscillations are observed</th>
</tr>
</thead>
<tbody>
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<td>$D_m$</td>
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<td>$\geq 1.38 \times 10^{-13} \text{ cm}^2 \text{s}^{-1}$</td>
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<td>$D_p$</td>
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<td>$\leq 3.70 \times 10^{-4} \text{ cm}$</td>
</tr>
<tr>
<td>$a$</td>
<td>$1.25 \times 10^{-6} \text{ cm} \text{s}^{-1}$</td>
<td>$1.92 \times 10^{-9} \text{ cm} \text{s}^{-1}$ to $8.33 \times 10^{-5} \text{ cm} \text{s}^{-1}$</td>
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</tbody>
</table>
Fig. 5  Plots of the total concentrations (in non-dimensional units) of hes1 mRNA (red) and Hes1 protein (blue) in (a) the nucleus and (b) the cytoplasm for the extended Hes1 model. The period of oscillation is approximately 120 minutes. Parameter values as per column 2 of Table 2.

Fig. 6  Graph showing nuclear to cytoplasmic (N/C) ratio of Hes1 protein plotted against $a$, the active transport speed. The plot shows that the nuclear to cytoplasmic ratio of Hes1 protein increases monotonically as $a$ is increased and tends to a limiting value.

We have examined the dependence of the nuclear to cytoplasmic ratio of Hes1 on the speed of active transport. Figure 6 shows 100 different values of $a$, plotted in increments of $2.08 \times 10^{-7}$ cm s$^{-1}$ (the sixth value, $1.25 \times 10^{-6}$ cm s$^{-1}$ is the default value used in simulations). All other parameter values are found in column 2 of Table 2. The nuclear to cytoplasmic ratio is obtained by taking the mean value of the total concentration of Hes1 protein in the nucleus over a 1000-minute time period and dividing it by the mean total value attained in the cytoplasm over the same time period. The plot shows that the nuclear to cytoplasmic ratio of Hes1 protein increases monotonically as $a$ is increased and tends to a limiting value. We leave these observations as predictions for experimentalists to corroborate.

Figures 7a and 7b show respectively how hes1 mRNA and Hes1 protein concentrations vary spatially within the cell from times $t = 150$ minutes to $t = 300$ minutes. At time $t = 150$ minutes, we see that nuclear hes1 mRNA levels are high and that there is also hes1 mRNA concentrated outside the nucleus. The presence of hes1 mRNA in the cytoplasm causes the production by translation of Hes1 protein, which is actively
transported towards the nucleus (times 150, 180). When Hes1 reaches the MTOC directly outside the nucleus, it is no longer actively transported but moves by diffusion alone. Hence Hes1 levels build up directly outside the nucleus. This build up is exacerbated by the nuclear membrane, across which Hes1 moves by slow diffusion. As levels of Hes1 rise in the nucleus, the transcription of hes1 mRNA is inhibited (times 180, 210). Without mRNA transcription, no new Hes1 protein can be created by translation. Hence levels of Hes1 fall throughout the cell by natural degradation (times 210, 240). In the absence of Hes1, mRNA transcription is no longer inhibited, and this process resumes (time 240 minutes). The cycle just described now repeats, and indeed the oscillatory period of two hours (120 minutes) is clear from comparing times 150 and 180 with times 270 and 300, respectively.
Spatial profiles for the original Hes1 model without an explicit nuclear membrane or active transport can be found in Figs. 6 and 7 in Sturrock et al. (2011). Although the time information in these figures in Sturrock et al. (2011) does not completely match the times in our new plots (Figs. 7a and 7b), a comparison is possible. The spatial profiles for hes1 mRNA are not changed qualitatively by our new extensions to the model—the local concentration in the nucleus still reaches a much higher peak than in the cytoplasm. However, the behaviour of Hes1 protein is changed. Instead of building up outside the nucleus as in Fig 7b, it spreads out across the cytoplasm in the absence of active transport and an explicit nuclear membrane (Fig. 7 in Sturrock et al. 2011).

2.2.2 Modelling Spatial Effects in the Nucleus

Until now we have assumed that the diffusion coefficients for all species in each compartment are equal. While this assumption helps reduce the number of parameters in the model, it may not be the most accurate approach. For example, it is known that proteins experience macromolecular crowding in the nucleus (Bancaud et al. 2009), so a different nuclear protein diffusion coefficient may be more appropriate. To this end, in Fig. 8 we present the results of simulations exploring the effect of varying the diffusion coefficients of the molecules in the nucleus. As shown in the plots, changing the diffusion coefficients causes a change in the amplitude and period of the oscillations.

We have also assumed that transcription of hes1 mRNA occurs throughout the nucleus (“uniformly distributed gene”). However, a more accurate way to model transcription would be to localise mRNA production to a smaller sub-region of the nucleus (“point source gene”). This can be achieved in the model by modifying (1) as follows:

\[
\frac{\partial [m_n]}{\partial t} = D_{m_n} \nabla^2 [m_n] + H_2(x, y) \left[ \frac{\alpha_m}{1 + ([p_n]/\hat{p})^h} \right] - \mu_m [m_n], \tag{17}
\]

where

\[
H_2(x, y) = \begin{cases} 
1 & \text{if } x^2 + y^2 \leq r^2, \\
0 & \text{if } x^2 + y^2 > r^2, 
\end{cases} \tag{18}
\]

and where \( r \) is the production zone radius. Simulation results from this modified model are presented in Fig. 9. The plots in Fig. 9(a) show the mRNA concentration in the nucleus over time as we reduce the production zone radius (\( r \)) and keep the mRNA production rate (\( \alpha_m \)) constant. Oscillatory dynamics are maintained until a critical value of the radius \( r \) is reached between 0.90 µm and 0.49 µm. The lower three plots show that there is a loss of oscillatory dynamics when the production zone is too small. The plots in Fig. 9(b) show the mRNA concentration in the nucleus over time as we decrease the production zone radius but increase the mRNA production rate (dividing the default value of \( \alpha_m \) by the area of the production zone). It is revealed in these plots that oscillatory dynamics can be maintained for smaller values of \( r \).
Fig. 8  Plots showing the effect on the concentration profiles of varying the mRNA and protein diffusion coefficients. In each row, the left plot shows the total concentrations in the cytoplasm, and the right plot shows the total concentrations in the nucleus (Hes1 protein in blue, hes1 mRNA in red). Plots in the first row correspond to the case where all four diffusion coefficients are different, i.e. $D_{pc} = 3.13 \times 10^{-11}$ cm$^2$s$^{-1}$, $D_{mc} = 2D_{pc}$, $D_{pn} = 5D_{pc}$, $D_{mn} = D_{pc}/10$. Plots in the second row correspond to the case where the nuclear diffusion coefficients are increased, i.e. $D_{pc} = D_{mc} = 3.13 \times 10^{-11}$ cm$^2$s$^{-1}$ and $D_{pn} = D_{mn} = 10D_{pc}$. Plots in the third row show the result of increasing the diffusion coefficients of mRNA compared with protein diffusion coefficients, i.e. $D_{pc} = D_{pn} = 3.13 \times 10^{-11}$ cm$^2$s$^{-1}$ and $D_{mc} = D_{mn} = 10D_{pc}$. All other parameter values are found in column 2 of Table 2

2.2.3 Convection as the Sole Transport Mechanism of Cytoplasmic Hes1 Protein

Our spatio-temporal modelling approach allows us to address questions which cannot be answered using ODE or DDE models. For example, we can investigate different ratios of active transport and diffusion such that sustained oscillatory dynamics occur in the extended Hes1 model. We begin to investigate this by decreasing the importance
Fig. 9  Plots showing the effect on mRNA concentration in the nucleus of localising transcription. In these simulations transcription (i.e. mRNA production) is localised to a region in the nucleus defined by (18). (a) The radius $r$ of the production zone is decreased while the mRNA production rate $\alpha_m$ is kept constant. As can be seen, oscillatory dynamics are present until $r$ becomes too small. (b) The radius $r$ of the production zone is decreased, but the mRNA production rate $\alpha_m$ is increased (dividing the baseline value of $\alpha_m$ by the area of the production zone). As can be observed, oscillatory dynamics are present for all values of $r$. Parameter values as per column 2 of Table 2.
of cytoplasmic protein diffusion relative to its active transport. Setting the diffusion coefficient $D_{pc}$ to zero, we were still able to find sustained oscillatory dynamics for a range of active transport rates $a$. Representative results are shown in Fig. 10 for two different values of $a$. Consistent with intuition, the plots shown in Fig. 10 show that a greater proportion of protein accumulates in the nucleus as the active transport rate $a$ is increased. Our results suggest that sustained oscillatory dynamics will occur as long as sufficient quantities of Hes1 protein reach the nucleus, regardless of the precise transport mechanism they use to reach it. We leave this result as a prediction of the model since we are not aware of any experiments which can demonstrate this. In the next section, we consider the opposite situation to that considered here, decreasing the importance of active transport relative to diffusion.

2.2.4 Microtubule Disruption

Microtubules are important in a diverse array of cellular functions, ranging from cell division to intracellular trafficking. Consequently microtubule-disrupting drugs are used in cancer therapy and are studied experimentally (Jordan and Wilson 2004; Kavallaris 2010; Carbonaro et al. 2011). Although we are not aware of microtubule-disrupting drugs being used on the Hes1 pathway, we shall consider the effect of such drugs in our extended Hes1 model and leave our observations as predictions. Clearly microtubule-disrupting drugs will disrupt active transport along microtubules, so we set the active transport rate $a$ equal to zero in our extended model and otherwise retain the parameter values in the second column of Table 2 (for convenience, the complete set of parameters is stated also in the second column of Table 4). Figure 11 shows the total concentrations for Hes1 protein and hes1 mRNA over time. The system no longer satisfies the predefined criteria for sustained oscillatory dynamics (at least five distinct peaks in the total concentration of the transcription factor in the nucleus);
Table 4  Parameter values used in the extended Hes1 model in the case where the active transport rate is set to zero, and ranges over which sustained oscillatory dynamics are observed

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value in simulations</th>
<th>Range over which oscillations are observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{ij}$</td>
<td>$3.13 \times 10^{-11}$ cm$^2$s$^{-1}$</td>
<td>$3.33 \times 10^{-11}$ cm$^2$s$^{-1}$ to $1.46 \times 10^{-9}$ cm$^2$s$^{-1}$</td>
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<td>$\alpha_m$</td>
<td>$6.25 \times 10^{-11}$ Ms$^{-1}$</td>
<td>$\geq 6.87 \times 10^{-11}$ Ms$^{-1}$</td>
</tr>
<tr>
<td>$\hat{p}$</td>
<td>$1.00 \times 10^{-9}$ M</td>
<td>$1.05 \times 10^{-9}$ M to $1.00 \times 10^{-5}$ M</td>
</tr>
<tr>
<td>$h$</td>
<td>5</td>
<td>$\geq 6$</td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>$1.25 \times 10^{-3}$ s$^{-1}$</td>
<td>$1.25 \times 10^{-4}$ s$^{-1}$ to $1.21 \times 10^{-3}$ s$^{-1}$</td>
</tr>
<tr>
<td>$\alpha_p$</td>
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<td>$\geq 0.0583$ s$^{-1}$</td>
</tr>
<tr>
<td>$\mu_p$</td>
<td>$1.25 \times 10^{-3}$ s$^{-1}$</td>
<td>$1.25 \times 10^{-4}$ s$^{-1}$ to $1.21 \times 10^{-3}$ s$^{-1}$</td>
</tr>
<tr>
<td>$D_m$</td>
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</tr>
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<td>$\geq 6.25 \times 10^{-12}$ cm$^2$s$^{-1}$</td>
</tr>
<tr>
<td>$d$</td>
<td>$1 \times 10^{-5}$ cm</td>
<td>$\leq 8.00 \times 10^{-6}$ cm</td>
</tr>
<tr>
<td>$a$</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>$l$</td>
<td>$6.32$ µm</td>
<td>Nuclear membrane (3 µm) to 6.24 µm</td>
</tr>
</tbody>
</table>

Fig. 11  Plots of the total concentrations of hes1 mRNA (red) and Hes1 protein (blue) in (a) the nucleus and (b) the cytoplasm for the extended Hes1 model in the absence of active transport. The concentrations exhibit damped oscillations. Parameter values as per column 2, Table 4

rather the oscillations are damped. This marks a qualitative change in the dynamics. In a general sense, this is an encouraging result—a qualitative change in dynamics is the type of response we might seek from drug therapy.

Figure 11 also shows quantitative changes in the dynamics. For example, a smaller proportion of Hes1 now enters the nucleus (relative to Fig. 5)—the total concentration of Hes1 in the nucleus is only roughly 1 % of that in the cytoplasm in Fig. 11. This reduction is to be expected since Hes1 is no longer actively transported towards the nucleus.

The damped nature of the oscillations can be seen in Fig. 12, which, like Fig. 7, shows the spatio-temporal evolution of hes1 mRNA and Hes1 protein from times
Fig. 12  Plots showing the spatio-temporal evolution of (a) hes1 mRNA and (b) Hes1 protein from times $t = 150$ to $t = 300$ minutes at 30 minute intervals for the extended Hes1 model in the absence of active transport. The concentrations exhibit damped oscillations in time and space. Parameter values as per column 2 of Table 4.

Although our set of parameter values (second column, Table 4) in the extended model without active transport did not yield sustained oscillatory dynamics, we found that by varying each parameter individually then such dynamics could occur. Ranges of values for each parameter such that sustained oscillations occur are stated in the third column of Table 4. Note that these ranges are narrower than those presented in...
Fig. 13 Images of (a) an osteosarcoma cell (U-2 OS) (reproduced with permission from Davidson 2011) and (b) an imported image of this cell with axes displayed in non-dimensional spatial units. (One non-dimensional spatial unit corresponds to 10 µm.) A third region directly outside the nucleus was added to the imported domain (shown as orange), the outer boundary of which represents the MTOC (cf. Fig. 4).

Tables 1 and 2 and that, unlike in Tables 1 and 2, they do not contain the experimental measurements for the parameters \( \mu_m \) and \( \mu_p \). Furthermore, unlike in Table 2, the experimental measurement for \( d \) is not contained in the range for \( d \) in Table 4.

2.2.5 Influence of Cell Shape

As mentioned in the Introduction, cell shape can influence intracellular signal transduction (Meyers et al. 2006; Neves et al. 2008). The influence of cell geometry on the Notch-Delta and NF-\( \kappa \)B pathways has recently been investigated by Terry and co-workers, who found through spatio-temporal modelling that oscillatory behaviour in these pathways is to some extent robust to changes in the shapes and relative sizes of the nucleus and cytoplasm (Terry et al. 2011; Terry and Chaplain 2011). We have performed numerous simulations to study the influence of cell shape on the extended Hes1 model, with parameters as in the second column of Table 2. We present some of these results in the Appendix. In general, we found that the qualitative behaviour of the extended Hes1 model is much more robust to variety in cell shape than the quantitative behaviour—oscillatory dynamics can be retained when the domain is altered whilst, for example, the proportion of Hes1 that enters the nucleus will change. Hence, to obtain quantitatively accurate results, we should use a domain that exactly matches a living cell.

We explore the effect of using a realistic cell shape in Figs. 14 and 15. For this, we have used an image of an osteosarcoma cell since the Hes1 pathway is known to play a critical role in the development of osteosarcomas (Zhang et al. 2008). The image of the osteosarcoma cell, taken from Davidson (2011), is shown in Fig. 13a, and the imported domain used for simulations is shown in Fig. 13b. An additional region was added to account for the MTOC.

The realistic cell domain does not change the solution qualitatively—oscillations are evident from the total concentration plots of Fig. 14. However, there are quanti-
Fig. 14  Plots of the total concentrations of hes1 mRNA (red) and Hes1 protein (blue) in (a) the nucleus and (b) the cytoplasm for the extended Hes1 model solved over an osteosarcoma cell domain as shown in Fig. 13b. The period of oscillation is approximately 112.5 minutes. Parameter values as per column 2 of Table 2.

Comparing Figs. 15 and 7 allows us to see why the total protein concentration in Fig. 14 is significantly less than that in Fig. 5. Whilst protein is produced uniformly around the nucleus in Fig. 7, this is not the case in the osteosarcoma cell in Fig. 15 because the nucleus is much nearer to the cell membrane and we have made the assumption that protein is produced a small distance from the nucleus. Protein is mainly produced in the osteosarcoma cell in the areas above and to the left of the nucleus, where there is space for this to occur.

3 The p53–Mdm2 Pathway

The p53 tumour suppressor protein is a well-established regulator of the cell cycle (Wang et al. 2010). In response to a variety of cellular stresses (e.g., DNA damage or oncogene activation), p53 is activated and translocates to the nucleus where it activates transcription of its target genes which can induce cell cycle arrest, senescence or apoptosis. One of the target genes for p53 is the gene for Mdm2. The Mdm2 oncogene product is a vital negative regulator of p53 function in cells. Mdm2 represses p53 function through two main mechanisms: (i) by promoting p53 ubiquitination and proteasomal degradation and, (ii) through direct inhibition of p53 transcriptional activity. This dual mechanism of inhibition is displayed in Fig. 16.
Fig. 15  Plots showing the spatio-temporal evolution of (a) hes1 mRNA and (b) Hes1 protein within the cell from times $t = 150$ to $t = 300$ minutes at 30 minute intervals for the extended Hes1 model solved over an osteosarcoma cell domain as shown in Fig. 13b. The concentrations exhibit oscillatory dynamics in both time and space. Parameter values as per column 2 of Table 2.

Fig. 16  Schematic of the p53–Mdm2 pathway. p53 mRNA produces p53 protein, which then upregulates Mdm2 mRNA expression. Mdm2 then enhances degradation of p53 (through ubiquitination) and inhibits the transcription of Mdm2 mRNA.
Experimental data have revealed that in response to gamma irradiation, p53 and Mdm2 concentrations exhibit oscillatory dynamics, both spatially and temporally (Geva-Zatorsky et al. 2006). These results have been confirmed by in vivo experiments (Hamstra et al. 2006), but the precise function of these oscillations is still under investigation (Zhang et al. 2007; Batchelor et al. 2009).

Many different mathematical modelling approaches have been adopted in order to capture and elucidate the oscillatory dynamics exhibited by the p53 pathway in response to DNA damage. Recent models have employed a combination of positive feedback loops and negative feedback loops in ODE metapopulation-like models (Ciliberto et al. 2005; Zhang et al. 2007). These models were the first to make the important distinction between nuclear and cytoplasmic concentrations. Other models have used time delays to replicate the observed oscillations (Mihalas et al. 2006; Ma et al. 2005; Batchelor et al. 2008), while some have explored the effects of stochasticity (Puszyński et al. 2008; Proctor and Gray 2008; Ouattara et al. 2010). A spatio-temporal model of the p53–Mdm2 pathway was recently developed in Sturrock et al. (2011).

3.1 The p53–Mdm2 Mathematical Model

We introduce a slightly modified version of the p53–Mdm2 PDE model presented in Sturrock et al. (2011). The modifications consist of introducing p53 mRNA to more accurately model translation of p53 in the cytoplasm, and we now account for Mdm2 inhibition of p53 transcriptional activity. We use the variables \([p53m], [p53], [Mdm2m], \) and \([Mdm2]\) to represent the concentrations of, respectively, p53 mRNA, p53 protein, Mdm2 mRNA, and Mdm2 protein. In keeping with the notation used for the Hes1 system, a subscript \(n\) denotes a nuclear concentration, and a subscript \(c\) denotes a cytoplasmic concentration.

As we did for the Hes1 model, we assume that all species are subject to diffusion, mRNA is produced only in the nucleus, and protein is produced only in the cytoplasm. Diffusion coefficients are denoted in a similar manner to the Hes1 system: a subscript indicates the localisation of the species, with \(n\) or \(c\) denoting a nuclear or cytoplasmic concentration and a subsubscript 1, 2, 3, or 4 referring to p53 mRNA, p53, Mdm2 mRNA, or Mdm2 respectively. We assume all species are subject to linear decay, with parameters \(\phi, \mu\) and \(\rho\) denoting mRNA decay, p53 protein decay, and Mdm2 protein decay, respectively. In addition, we assume that p53 undergoes Mdm2-dependent degradation in both the nucleus and cytoplasm. This is consistent with experimental data which shows that co-compartmentalisation of p53 and Mdm2 results in Mdm2 dependent degradation of p53 (Xirodimas et al. 2001). We assume that this degradation term is equal to a linear decay term with parameter \(\nu\), scaled by a bounded monotonically increasing function of Mdm2 protein concentration with Hill coefficient \(h_1\) and activation threshold \(\hat{M}_{\text{dm2}}\). We make the assumption that p53 mRNA is produced at a constant rate \(\zeta\) and Mdm2 mRNA is produced at a constant rate \(\alpha\). Further, we assume Mdm2 mRNA undergoes nuclear p53 dependent production (taking the form of a Hill-like function), with rate \(\eta\), Hill coefficient \(h_2\), and
critical concentration $\hat{p}53$. This enhanced production term is also assumed to decrease as nuclear Mdm2 protein levels increase, with parameter $\theta$. This assumption takes into account the fact that Mdm2 protein inhibits the transcriptional activity of p53 (Thut et al. 1997). Finally, we assume that protein production occurs a small distance outside the nucleus (as in the case of the Hes1 model) and is dependent on the relevant concentration of mRNA, occurring at rate $\beta$ for p53 protein and $\gamma$ for Mdm2 protein.

The complete system of equations is given by:

$$\frac{\partial [p53_m]}{\partial t} = D_{n1} \nabla^2 [p53_m] + \zeta - \phi[p53_m],$$

$$\frac{\partial [p53_c]}{\partial t} = D_{c1} \nabla^2 [p53_c] - \phi[p53_c],$$

$$\frac{\partial [p53_e]}{\partial t} = D_{c2} \nabla^2 [p53_e] + H_1(x, y) \beta[p53_c] - \left(\mu + \nu \left(\frac{[Mdm2_c]^{h_1}}{Mdm2_c^{h_1} + [Mdm2_c]^{h_1}}\right)\right)[p53_e],$$

$$\frac{\partial [p53_n]}{\partial t} = D_{n2} \nabla^2 [p53_n] - \left(\mu + \nu \left(\frac{[Mdm2_n]^{h_1}}{Mdm2_n^{h_1} + [Mdm2_n]^{h_1}}\right)\right)[p53_n],$$

$$\frac{\partial [Mdm2_m]}{\partial t} = D_{n3} \nabla^2 [Mdm2_m] + \alpha + \eta \left(\frac{[p53_n]^{h_2}}{(p53 + [Mdm2_n]/\theta)^{h_2} + [p53_n]^{h_2}}\right) - \phi[Mdm2_m],$$

$$\frac{\partial [Mdm2_c]}{\partial t} = D_{c3} \nabla^2 [Mdm2_c] - \phi[Mdm2_c],$$

$$\frac{\partial [Mdm2_c]}{\partial t} = D_{c4} \nabla^2 [Mdm2_c] + H_1(x, y) \gamma[Mdm2_m] - \rho[Mdm2_c],$$

$$\frac{\partial [Mdm2_n]}{\partial t} = D_{n4} \nabla^2 [Mdm2_n] - \rho[Mdm2_n],$$

where $H_1(x, y)$ is the function controlling cytoplasmic protein production in ribosomes defined in (5).

We apply zero initial conditions, zero-flux boundary conditions at the cell membrane, and continuity of flux boundary conditions across the nuclear membrane:

$$[p53_m] = [p53_c] = [p53_e] = [p53_n] = [Mdm2_m] = [Mdm2_c]$$

$$= [Mdm2_n] = [Mdm2_c] = 0 \text{ at } t = 0,$$

$$D_{n1} \frac{\partial [p53_m]}{\partial n} = D_{c1} \frac{\partial [p53_c]}{\partial n}, \quad \text{and}$$

$$[p53_m] = [p53_c] \text{ at nuclear membrane,}$$
\[ D_{n2} \frac{\partial [p53_n]}{\partial n} = D_{c2} \frac{\partial [p53_c]}{\partial n}, \quad \text{and} \]
\[ [p53_n] = [p53_c] \quad \text{at nuclear membrane,} \]

\[ D_{n3} \frac{\partial [Mdm2m_n]}{\partial n} = D_{c3} \frac{\partial [Mdm2m_c]}{\partial n}, \quad \text{and} \]
\[ [Mdm2m_n] = [Mdm2m_c] \quad \text{at nuclear membrane,} \]

\[ D_{n4} \frac{\partial [Mdm2n]}{\partial n} = D_{c4} \frac{\partial [Mdm2c]}{\partial n}, \quad \text{and} \]
\[ [Mdm2n] = [Mdm2c] \quad \text{at nuclear membrane,} \]

\[ \frac{\partial [p53mc]}{\partial n} = 0 \quad \text{at cell membrane,} \]
\[ \frac{\partial [p53c]}{\partial n} = 0 \quad \text{at cell membrane,} \]
\[ \frac{\partial [Mdm2mc]}{\partial n} = 0 \quad \text{at cell membrane,} \]
\[ \frac{\partial [Mdm2c]}{\partial n} = 0 \quad \text{at cell membrane.} \]

Given that the PDE system defined by (19)–(26) subject to conditions (27)–(35) is a modified version of the p53–Mdm2 model in Sturrock et al. (2011), it has not been explored numerically before. In the interests of making quantitative predictions and since the p53 pathway is known to play a role in the development of osteosarcomas (Diller et al. 1990), we choose the imported shape of an osteosarcoma cell shown in Fig. 13b as our domain. Our objective is to study sustained oscillatory dynamics, so we must find non-dimensional parameter values such that our model yields such dynamics. Nearly all of the parameters in our new modified model are contained in the original p53–Mdm2 model in Sturrock et al. (2011), which has already been studied in the context of oscillations. Hence, for these parameters, we choose the non-dimensional values used for the original model, which are stated in (60) in Sturrock et al. (2011). The remaining parameters are \( \zeta \) and \( \theta \), for which we have found appropriate values by a simulation study. From our non-dimensional parameter values, we have calculated dimensional values and these are stated in the third column of Table 6. Details regarding non-dimensionalisation, and the calculation of dimensional parameter values can be found in the Appendix. As in Sect. 2.1, all nuclear and cytoplasmic diffusion coefficients are equal to each other, and we retain the notation \( D_{ij} \) to indicate diffusion of species \( i \) (mRNA or protein) in location \( j \) (nucleus or cytoplasm).

We have found ranges of values for all of the parameters in our new modified p53–Mdm2 model such that it exhibits sustained oscillatory dynamics, where (as in Sect. 2.1) we define such dynamics as at least five distinct peaks in the total concentration of the transcription factor (in this case, p53) in the nucleus. These ranges are given in the fourth column in Table 5. Once again, to find the range for each parame-
Table 5 Description of parameters in the p53–Mdm2 model defined in Sect. 3.1, values used in simulations, and ranges over which sustained oscillatory dynamics are observed

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value in simulations</th>
<th>Range over which oscillations are observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{ij}$</td>
<td>Diffusion coefficient of species $i$ in compartment $j$</td>
<td>$3.00 \times 10^{-11} \text{ cm}^2 \text{s}^{-1}$</td>
<td>$1.00 \times 10^{-11} \text{ cm}^2 \text{s}^{-1}$ to $1.67 \times 10^{-8} \text{ cm}^2 \text{s}^{-1}$</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>Basal rate of p53 mRNA transcription</td>
<td>$2.92 \times 10^{-10} \text{ M s}^{-1}$</td>
<td>$\geq 5.83 \times 10^{-11} \text{ M s}^{-1}$</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Degradation rate of mRNA</td>
<td>$5.83 \times 10^{-4} \text{ s}^{-1}$</td>
<td>$1.00 \times 10^{-4} \text{ s}^{-1}$ to $1.10 \times 10^{-3} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Translation rate of p53</td>
<td>$0.33 \text{ s}^{-1}$</td>
<td>$\leq 5.13 \times 10^{-2} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Degradation rate of p53</td>
<td>$1.00 \times 10^{-4} \text{ s}^{-1}$</td>
<td>$\leq 4.33 \times 10^{-4} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$\nu$</td>
<td>Mdm2 dependent degradation of p53</td>
<td>$3.33 \times 10^{-2} \text{ s}^{-1}$</td>
<td>$1.67 \times 10^{-3} \text{ s}^{-1}$ to $3.33 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$h_1$</td>
<td>Hill coefficient for Mdm2 dependent degradation of p53</td>
<td>2</td>
<td>$\geq 1$</td>
</tr>
<tr>
<td>$\tilde{M}_{\text{Mdm2}}$</td>
<td>Activation threshold for Mdm2 dependent degradation of p53</td>
<td>$3.20 \times 10^{-5} \text{ M}$</td>
<td>$3.20 \times 10^{-6} \text{ s}^{-1}$ to $2.10 \times 10^{-4} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Basal rate of Mdm2 mRNA transcription</td>
<td>$2.92 \times 10^{-11} \text{ M s}^{-1}$</td>
<td>$\leq 2.25 \times 10^{-10} \text{ M s}^{-1}$</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Maximal p53 dependent transcription of Mdm2 mRNA</td>
<td>$1.67 \times 10^{-9} \text{ M s}^{-1}$</td>
<td>$\geq 2.08 \times 10^{-10} \text{ M s}^{-1}$</td>
</tr>
<tr>
<td>$h_2$</td>
<td>Hill coefficient for p53 dependent transcription</td>
<td>4</td>
<td>$\geq 1$</td>
</tr>
<tr>
<td>$\tilde{p}_{53}$</td>
<td>Threshold parameter of p53</td>
<td>$2.50 \times 10^{-6} \text{ M}$</td>
<td>$\leq 1.65 \times 10^{-5} \text{ M}$</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Mdm2 inhibition of p53 transcription</td>
<td>4.00</td>
<td>$\geq 15.60 \times 10^{-3}$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Translation rate of Mdm2</td>
<td>$0.67 \text{ s}^{-1}$</td>
<td>$\geq 0.10 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Degradation rate of Mdm2</td>
<td>$8.33 \times 10^{-4} \text{ s}^{-1}$</td>
<td>$1.33 \times 10^{-4} \text{ s}^{-1}$ to $7.00 \times 10^{-3} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$l$</td>
<td>Minimum radial distance of translation from centre of nucleus</td>
<td>$6.32 \mu\text{m}$</td>
<td>$3.46 \mu\text{m}$ to $9.27 \mu\text{m}$</td>
</tr>
</tbody>
</table>

After, we varied it whilst holding all the other parameters fixed at their “default” values, the dimensional versions of which are stated in the third column of Table 5.

3.1.1 p53–Mdm2 Model Parameter Values

The p53–Mdm2 model permits oscillatory dynamics for a wide range of diffusion coefficients, which include the experimentally measured values of Matsuda et al. (2008), Seksek et al. (1997). We note that this diffusion coefficient can be even larger when more spatial structure within the cell is included (see Table 6, Sect. 3.2.1).

Only two of the parameters in Table 5 have been measured experimentally, namely the degradation rate $\mu$ of p53 protein and the degradation rate $\rho$ of Mdm2 protein. According to Finlay (1993), these degradation rates are approximately $5.85 \times 10^{-4} \text{ s}^{-1}$ for both p53 and Mdm2. This value lies within the ranges calculated which produce oscillatory dynamics (see table entries for $\mu$ and $\rho$). Assuming that the decay rates of
p53 mRNA and Mdm2 mRNA are of roughly the same order as the decay rate of hes1 mRNA, which has been estimated experimentally at $4.83 \times 10^{-4}$ s$^{-1}$ (Hirata et al. 2002), then the range presented for \( \phi \) is in agreement with experimentally measured values. To calculate the value and range for the parameter \( l \), defined in Table 5 as the minimum radial distance of translation from the centre of the nucleus, we took the centre of the nucleus to be the origin in the non-dimensional domain in Fig. 13b. Interestingly, we find that protein translation must begin a small distance from the nuclear membrane for this case. Our ranges of values for the remaining parameters in Table 5 are consistent with the values found in the modelling literature, where analogous parameters exist (Proctor and Gray 2008; Ciliberto et al. 2005; Puszyński et al. 2008; Geva-Zatorsky et al. 2006).

3.2 Extended p53–Mdm2 Model

We now extend the p53–Mdm2 model defined in Sect. 3.1 to include a nuclear membrane and active transport. The importance of modelling the nuclear membrane explicitly has been made clear in Sect. 2.2, but, in terms of p53 specifically, it is worth noting that p53 nucleocytoplasmic transport is known to be tightly regulated and that disruption to this transport can play a role in tumorigenesis (Ryan et al. 2001). We define the explicit nuclear membrane boundary conditions in a similar manner to the Hes1 model in Sect. 2.2. Thus, recalling our notation from Sect. 3.1 that \( D_{ij} \) indicates diffusion of species \( i \) (mRNA or protein) in location \( j \) (nucleus or cytoplasm), and still assuming that \( D_{ij} \) is constant (independent of \( i \) and \( j \)), we define mRNA and protein diffusion coefficients in the nuclear membrane as, respectively, \( D_m = D_{ij}/5 \) and \( D_p = D_{ij}/15 \) to reflect slow mRNA diffusion across the nuclear membrane and even slower protein diffusion, and we replace boundary conditions (28)–(31) with boundary conditions appropriate for a permeable thin boundary layer of thickness \( d \) defined by:

\[
D_{n1} \frac{\partial [p53m_n]}{\partial n} = \frac{D_m ([p53m_n] - [p53m_c])}{d}, \quad (36)
\]
\[
D_{c1} \frac{\partial [p53m_c]}{\partial n} = \frac{D_m ([p53m_c] - [p53m_n])}{d}, \quad (37)
\]
\[
D_{n2} \frac{\partial [p53c_n]}{\partial n} = \frac{D_p ([p53c_n] - [p53c])}{d}, \quad (38)
\]
\[
D_{c2} \frac{\partial [p53c_c]}{\partial n} = \frac{D_p ([p53c_c] - [p53c_n])}{d}, \quad (39)
\]
\[
D_{n3} \frac{\partial [Mdm2m_n]}{\partial n} = \frac{D_m ([Mdm2m_n] - [Mdm2m_c])}{d}, \quad (40)
\]
\[
D_{c3} \frac{\partial [Mdm2m_c]}{\partial n} = \frac{D_m ([Mdm2m_c] - [Mdm2m_n])}{d}, \quad (41)
\]
\[
D_{n4} \frac{\partial [Mdm2c_n]}{\partial n} = \frac{D_p ([Mdm2c_n] - [Mdm2c])}{d}, \quad (42)
\]
\[
D_{c4} \frac{\partial [Mdm2c_c]}{\partial n} = \frac{D_p ([Mdm2c_c] - [Mdm2c_n])}{d}. \quad (43)
\]
In terms of active transport, it is known that p53 is shuttled towards the nucleus along microtubules (O’Brate and Giannakakou 2003; Lomakina and Nadezhdina 2010). Although there is no direct evidence for Mdm2 transport along microtubules, there is evidence to suggest that Mdm2 can be actively transported to the nucleus (Mayo and Donner 2001). Therefore, as we did in Sect. 2.2 for the Hes1 model, we shall include convection terms in the cytoplasmic protein equations to account for active transport, which changes (21) and (25) to the following:

\[
\frac{\partial [p53_c]}{\partial t} = D_c \nabla^2 [p53_c] - \nabla \cdot \left( a [p53_c] \right) + H_1(x, y) \beta [p53_m_c]
\]

\[
- \left( \mu + v \left( \frac{[Mdm2_c]^{a_1}}{Mdm2^{a_1} + [Mdm2_c]^{a_1}} \right) \right) [p53_c],
\]

(44)

\[
\frac{\partial [Mdm2_c]}{\partial t} = D_c \nabla^2 [Mdm2_c] - \nabla \cdot \left( a [Mdm2_c] \right) + \gamma [Mdm2_m_c] - \rho [Mdm2_c],
\]

(45)

where the convective velocity \( a \) is defined as in (16) and is plotted in Fig. 3. As we did for the extended Hes1 model, we assume that convection does not occur in the region between the MTOC and the nuclear membrane (the orange region in Fig. 13b). Hence, in this region, (21) and (25) apply.

### 3.2.1 Simulation Results for Extended p53–Mdm2 Model

We performed simulations of the extended p53–Mdm2 model given by (19)–(26) and (44)–(45) subject to conditions (27) and (32)–(43). We retained the parameter values used to simulate the p53–Mdm2 model in Sect. 3.1 and for the additional parameters introduced by extending the model we chose values to give numerically stable sustained oscillations. Our parameter values are summarised in the second column of Table 6. Parameter ranges such that the extended model exhibits sustained oscillatory dynamics were found and are stated in the third column of Table 6.

Notice that most of the ranges in Table 6 are wider than those in Table 5, and in particular this is true for the diffusion coefficient. Hence, as we found for the Hes1 model, extending the p53–Mdm2 model to include a nuclear membrane and active transport makes it a more robust oscillator. Our rate of active transport in the second column of Table 6 is similar to the rate of active transport used in the Hes1 model in Table 2. However, notice that, unlike in Table 2, our range of values for the active transport rate in Table 6 includes zero. Hence active transport is not needed for sustained oscillatory dynamics in the extended p53–Mdm2 model (see Sect. 3.2.2 below). The parameter \( l \) permits oscillations over a larger range than in Table 5 but still does not permit sustained oscillations when translation occurs too close to the nucleus.

Figure 17 shows how the total concentrations of the variables in the extended p53–Mdm2 model vary over time in both the nucleus and cytoplasm. The model has changed significantly from that which was presented in Sturrock et al. (2011) in terms of the model equations, boundary conditions, and domain, but the solution still exhibits oscillatory dynamics (compare Fig. 17 with Figs. 17 and 18).
in Sturrock et al. 2011). However, there are numerous quantitative differences in the solution. For instance, a far larger proportion of the p53 and Mdm2 proteins now enters the nucleus, on account of being actively transported towards it and despite the barrier of slower diffusion across the nuclear membrane. To be more specific, Figs. 17 and 18 in Sturrock et al. (2011) show that peaks in nuclear p53 total concentration are approximately 8% the height of peaks in cytoplasmic p53 total concentration, whereas in Fig. 17 in our new results this has changed to 33%. For Mdm2, the change is from approximately 2.5% to 33%. The peaks in p53 total nuclear concentration are taller and narrower in our new results, exhibiting pulsatile-like dynamics and dropping to zero between consecutive peaks. Such dynamics are consistent with recent experimental data showing that, in response to DNA damage, p53 exhibits sharp pulses (Batchelor et al. 2009; Loewer et al. 2010). Note that p53 mRNA does not exhibit oscillations in Fig. 17 since it is not involved in a negative feedback loop and is not coupled to any other equations.

In Fig. 18 we show spatial profiles for p53 and Mdm2 from times $t = 240$ minutes to $t = 540$ minutes at 60 minute intervals. At $t = 240$ minutes, it can be observed that p53 has accumulated in the cytoplasm and nucleus. In the nucleus it upregulates Mdm2 mRNA transcription, which leads to increased production of Mdm2 in the cytoplasm ($t = 300$). Mdm2 enhances degradation of p53, both in the cyto-

### Table 6 Parameter values used in the extended p53–Mdm2 model and ranges over which sustained oscillatory dynamics are observed

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value in simulations</th>
<th>Range over which oscillations are observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{ij}$</td>
<td>$3.00 \times 10^{-11}$ cm$^2$ s$^{-1}$</td>
<td>$3.67 \times 10^{-12}$ cm$^2$ s$^{-1}$ to $5.33 \times 10^{-8}$ cm$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>$2.92 \times 10^{-10}$ M s$^{-1}$</td>
<td>$\geq 9.12 \times 10^{-12}$ M s$^{-1}$</td>
</tr>
<tr>
<td>$\phi$</td>
<td>$5.83 \times 10^{-4}$ s$^{-1}$</td>
<td>$2.00 \times 10^{-4}$ s$^{-1}$ to $1.87 \times 10^{-4}$ s$^{-1}$</td>
</tr>
<tr>
<td>$\beta$</td>
<td>$0.33$ s$^{-1}$</td>
<td>$\geq 9.33 \times 10^{-3}$ s$^{-1}$</td>
</tr>
<tr>
<td>$\mu$</td>
<td>$1.00 \times 10^{-4}$ s$^{-1}$</td>
<td>$\leq 1.67 \times 10^{-3}$ s$^{-1}$</td>
</tr>
<tr>
<td>$\nu$</td>
<td>$3.33 \times 10^{-2}$ s$^{-1}$</td>
<td>$6.67 \times 10^{-4}$ s$^{-1}$ to $1.17$ s$^{-1}$</td>
</tr>
<tr>
<td>$h_1$</td>
<td>2</td>
<td>$\geq 1$</td>
</tr>
<tr>
<td>$\bar{p}_{53}$</td>
<td>$3.2 \times 10^{-5}$ M</td>
<td>$5.60 \times 10^{-6}$ M to $8.00 \times 10^{-4}$ M</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>$2.92 \times 10^{-11}$ M s$^{-1}$</td>
<td>$\leq 1.50 \times 10^{-10}$ M s$^{-1}$</td>
</tr>
<tr>
<td>$\eta$</td>
<td>$1.67 \times 10^{-9}$ M s$^{-1}$</td>
<td>$\geq 1.04 \times 10^{-10}$ M s$^{-1}$</td>
</tr>
<tr>
<td>$h_2$</td>
<td>4</td>
<td>$\geq 1$</td>
</tr>
<tr>
<td>$\bar{M}_{Mdm2}$</td>
<td>$2.50 \times 10^{-6}$ M</td>
<td>$\leq 1.13 \times 10^{-4}$ M</td>
</tr>
<tr>
<td>$\theta$</td>
<td>4.00</td>
<td>$\geq 1.56 \times 10^{-2}$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>$0.67$ s$^{-1}$</td>
<td>$\geq 0.05$ s$^{-1}$</td>
</tr>
<tr>
<td>$\rho$</td>
<td>$8.33 \times 10^{-4}$ s$^{-1}$</td>
<td>$2.33 \times 10^{-4}$ s$^{-1}$ to $3.67 \times 10^{-3}$ s$^{-1}$</td>
</tr>
<tr>
<td>$D_m$</td>
<td>$6.00 \times 10^{-12}$ cm$^2$ s$^{-1}$</td>
<td>$\geq 2.22 \times 10^{-14}$ cm$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>$D_p$</td>
<td>$2.00 \times 10^{-12}$ cm$^2$ s$^{-1}$</td>
<td>$\geq 7.43 \times 10^{-15}$ cm$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>$d$</td>
<td>$1.00 \times 10^{-5}$ cm</td>
<td>$\leq 1.00 \times 10^{-3}$ cm</td>
</tr>
<tr>
<td>$a$</td>
<td>$1.00 \times 10^{-6}$ cm s$^{-1}$</td>
<td>$\leq 5.83 \times 10^{-5}$ cm s$^{-1}$</td>
</tr>
<tr>
<td>$l$</td>
<td>$6.32 \mu$m</td>
<td>$3.87 \mu$m to $11.8 \mu$m</td>
</tr>
</tbody>
</table>
plasm and in the nucleus. In particular, since Mdm2 is actively transported to the nucleus, Mdm2-dependent degradation of p53 is sufficiently strong to eradicate p53 there \((t = 360)\). Mdm2 levels fall through natural degradation, which frees p53 from Mdm2 dependent degradation and allows levels of p53 to rise in the cytoplasm. Levels of p53 quickly then rise in the nucleus through active transport \((t = 420)\). The process just described now repeats, producing oscillatory dynamics. The 180 minute period of oscillations is clear from Fig. 18. It is also clear that the nuclear membrane retards the nuclear entry of p53 and Mdm2—the local concentrations reach their highest levels in or next to the nuclear membrane. This result reinforces the idea, discussed in Gasiorowski and Dean (2003), Chahine and Pierce (2009), that the nuclear pore complex is an attractive site for delivering chemotherapeutic drugs to disrupt or enhance intracellular signalling. We also note that we have created “computational animations” of the numerical solution of the extended p53–Mdm2 model. Our computational animations are qualitatively similar to the experimental results obtained by Lahav et al. (2004) (supporting online material) employing fluorescent fusion proteins to visualise the protein concentration levels inside single cells—see the Supporting Information section below.

As was the case with the extended Hes1 model in Sect. 2.2.3, we found in the extended p53–Mdm2 model that oscillatory dynamics could occur even when the cytoplasmic protein diffusion coefficients were all set to zero (results not shown). In other words, it is possible to observe sustained oscillatory dynamics when proteins are transported to the nucleus by convection alone.

### 3.2.2 Microtubule Disruption

In Sect. 2.2.4 we mentioned that microtubules are seen as an attractive target for chemotherapeutic drugs. Hence, we now consider the effect of such drugs in our extended p53–Mdm2 model. The effect of such drugs will be to disrupt active transport,
Fig. 18 Plots showing the spatio-temporal evolution of (a) p53 and (b) Mdm2 within the osteosarcoma cell domain from times $t = 240$ to $t = 540$ minutes at 60 minute intervals for the extended p53–Mdm2 model. The concentrations exhibit oscillatory dynamics in both time and space. Parameter values as per column 2 of Table 6.

and therefore we set the active transport rate $a$ equal to zero in our extended model. All other parameter values are as per the second column of Table 6 (for convenience, the complete set of parameters is also stated in the second column of Table 7 below).

Figure 19 shows the total concentrations for all model species over time. Sustained oscillatory dynamics can be seen, but the oscillations are now smoother than when active transport was permitted, levels of nuclear p53 no longer drop to zero between successive peaks, the amplitude of p53 oscillations has grown enormously relative to Mdm2 oscillations in both the nucleus and the cytoplasm, and the oscillatory period has significantly increased (compare Fig. 19 with Fig. 17). There are also reductions in the overall amounts of nuclear p53 and nuclear Mdm2. For p53, peaks in total nuclear concentration are approximately 6% the height of peaks in total cytoplasmic...
Plots of the total concentrations of p53 mRNA (black), p53 (blue), Mdm2 mRNA (green) and Mdm2 (red) in (a) the nucleus and (b) the cytoplasm, for the extended p53–Mdm2 model in the absence of active transport. The period of oscillation is approximately 242.5 minutes. Parameter values as per column 2 of Table 7

concentration in Fig. 19, reduced from 33% in Fig. 17, while for Mdm2, the reduction is from 33% in Fig. 17 to 2.5% in Fig. 19. These latter findings are consistent with in vivo experiments showing that the microtubule-depolymerizing agent nocodazole causes levels of nuclear p53 to fall (Roth et al. 2007), and is also consistent with experiments showing that the treatment of cells with microtubule-disrupting agents before subjecting these cells to DNA damage causes both nuclear p53 and nuclear Mdm2 levels to fall (Giannakakou et al. 2000).

Figure 20 shows spatial profiles for p53 and Mdm2 from times \( t = 240 \) minutes to \( t = 540 \) minutes at 60 minute intervals. These proteins are produced in the cytoplasm by the process of translation, a process which we earlier assumed to occur at least some minimal distance from the nuclear membrane. This assumption has a clear impact on the local concentrations of p53 and Mdm2 in Fig. 20. New production of p53 and Mdm2 is maximal at this minimal distance where, by our assumptions, p53 mRNA and Mdm2 mRNA molecules diffusing outwards from the nucleus will first encounter ribosomes. Newly synthesised p53 and Mdm2 diffuse outwards into the cytoplasm, reaching the cell membrane in many places.

The spatial profiles in Fig. 20 are quite different to those in Fig. 18 where active transport was permitted and forced newly synthesised p53 and Mdm2 to rapidly translocate towards the nucleus. In the absence of directed transport towards the nucleus, the local concentrations of p53 and Mdm2 within or next to the nuclear membrane are hugely reduced. There is a chemotherapeutic implication. Chemotherapeutic drugs are often used in combination, a practise known as combination chemotherapy (Ferrari and Palmerini 2007; Robati et al. 2008). The biggest advantage to this practise is that it minimises the chances of resistance developing to any one agent. Drugs which target proteins at the nuclear membrane will be ineffective if little of the protein reaches the nuclear membrane, but Fig. 18 shows that microtubule-disrupting drugs may cause comparatively little of the protein to reach the nuclear membrane. Hence, the effectiveness of drugs designed to act at nuclear pore complexes may
be compromised by microtubule-disrupting drugs, and the combination of these two types of drug may not always represent an optimal treatment strategy.

Table 7 contains ranges of parameter values which permit oscillatory dynamics in the extended p53–Mdm2 model with no active transport. These ranges are narrower than those in Table 5, where there was no active transport and no explicit nuclear membrane, and are also narrower than the ranges in Table 6 where there was both active transport and an explicit nuclear membrane. These results are consistent with our findings for the Hes1 model in Sect. 2.2.4. The parameter range for $l$ now allows for protein translation to occur directly outside the nucleus. This is a result of the nuclear membrane slowing the entry of p53 to the nucleus, preventing Mdm2 levels from spiking too quickly.
3.2.3 Proteasome Inhibition

The proteasome is a large proteolytic protein complex found in all eukaryotic cells that is the primary site for degradation of most intracellular proteins (Alberts et al. 2008). The proteolytic activities of the proteasome can be inhibited by the class of drugs known as proteasome inhibitors (Orlowski and Kuhn 2008). It is known that exposing cells to proteasome inhibitors results in increased levels of p53 and Mdm2. In particular, it was shown in Maki et al. (1996) that gamma-irradiated cells treated with the proteasome inhibitor MG115 caused increased expression of p53. More recently, experiments conducted by Xirodimas et al. (2001) revealed that by treating cells with proteasome inhibitor MG132, both p53 and Mdm2 levels increased. Furthermore, both proteins localised in the nucleus. To model this effect, we decrease the protein degradation parameters, $\mu$, $v$, and $\rho$ by a factor $\lambda$, the inhibition factor. All other parameter values used for the simulations are as detailed in Table 6, but we divide $\mu$, $v$, and $\rho$ by $\lambda = 300$ so that their values become:

$$\mu = 3.33 \times 10^{-7} \text{s}^{-1}, \quad v = 1.11 \times 10^{-4} \text{s}^{-1}, \quad \rho = 2.78 \times 10^{-6} \text{s}^{-1}.$$  (46)

We do not reduce these protein degradation parameters to zero because proteasome inhibitors are not 100% efficient (Lightcap et al. 2000).
Fig. 21  Plots of the total concentrations of p53 mRNA (black), p53 (blue), Mdm2 mRNA (green) and Mdm2 (red) in (a) the nucleus and (b) the cytoplasm, for the extended p53–Mdm2 model. We also note that the proteins in the cytoplasm are largely concentrated in the region between the nucleus and the MTOC, i.e. very close to the nucleus, as can be seen in Fig. 22. Parameter values as per column 2 of Table 6, with the exception of parameters $\mu$, $\nu$, and $\rho$ which are specified in (46). The total concentrations of p53 and Mdm2 continue to increase over the 1500 minute time interval and accumulate mainly in the nuclear compartment.

In Fig. 21 we can see how the decrease in protein degradation parameters has affected the total concentrations of the variables in the p53–Mdm2 model including nuclear membrane and active transport. The system no longer exhibits oscillatory dynamics, but instead p53 and Mdm2 levels increase monotonically, quickly exceeding the levels in Fig. 17 where there was no proteasome inhibition. We can now see the level of protein in the nucleus exceeds that of the cytoplasm. Furthermore, the proteins in the cytoplasm are actually largely concentrated in the region between the nucleus and the MTOC, i.e. very close to the nucleus. This is reflected in the spatial plots presented in Fig. 22, where we can see high local concentrations of p53 and Mdm2 in the nucleus at time $t = 1500$ minutes, which accurately reflects the experimental findings of Xirodimas et al. (2001), Maki et al. (1996). The reason p53 and Mdm2 accumulate in the nucleus is due to active transport directing both species towards the nucleus. Mdm2 levels rapidly increase because the degradation rate of Mdm2 protein is decreased. Notice that p53 levels also increase, but not as rapidly as Mdm2. As there is a higher concentration of Mdm2 in the cell, this increases the likelihood of p53 being degraded via Mdm2 (although $\nu$ has been decreased, it is not zero). Mdm2 mRNA levels remain low in spite of increased levels of p53 as a result of Mdm2 protein directly inhibiting p53 transcriptional activity. p53 mRNA levels are unaffected by this numerical experiment.

Combination chemotherapy was mentioned in Sect. 3.2.2, and we noted that the combination of microtubule-disrupting drugs and drugs designed to act at nuclear pore complexes may not always represent an optimal treatment strategy. We can now add to this discussion. Figure 22 suggests that when proteins that are actively transported towards the nucleus are influenced by proteasome inhibitor drugs, their local concentration will rise significantly at the nuclear membrane. Hence the combination
Fig. 22 Plots showing the spatial distribution of (a) p53 and (b) Mdm2 within the osteosarcoma cell domain of Fig. 13b at time $t = 1500$ minutes, for the extended p53–Mdm2 model. The concentrations of p53 and Mdm2 are localised mainly in the nucleus and between the nuclear membrane and the MTOC. Parameter values as per column 2 of Table 6, with the exception of parameters $\mu$, $\nu$, and $\rho$ which are specified in (46).

![Fig. 22](image)

Fig. 23 Plots of the nuclear to cytoplasmic (N/C) ratio against the inhibition factor $\lambda$ for (a) p53 and (b) Mdm2. Values of $\lambda$ are plotted in increments of 50, starting with 1 and ending with 1001. Parameter values are found in column 2 of Table 6, with the exception of parameters $\mu$, $\nu$, and $\rho$ which are reduced by a factor $\lambda$.

![Fig. 23](image)

of drugs designed to act at nuclear pore complexes with proteasome inhibitor drugs may represent a potentially fruitful avenue for new chemotherapeutic experimental studies. We anticipate that extensions to our spatio-temporal modelling approach will allow us to make more specific suggestions for new experiments in due course.

In Fig. 23 we explore the relationship between the nuclear to cytoplasmic ratio of protein and the inhibition factor, $\lambda$. We achieved this by first calculating the total concentrations of nuclear and cytoplasmic protein over a 1500 minute time period for different values of $\lambda$. We then calculated the mean of these total concentrations.
and divided the nuclear mean by the cytoplasmic mean. Finally, we plotted this ratio against the value of \( \lambda \). As can be seen from the plots, the nuclear to cytoplasmic ratio of p53 monotonically increases as \( \lambda \) is increased, whereas the nuclear to cytoplasmic ratio of Mdm2 saturates once \( \lambda \) reaches a value of approximately 150. From Fig. 23, we can make the quantitative prediction that the proteasome inhibitor must effectively reduce the degradation rates by a factor of 200 before more p53 and Mdm2 will accumulate in the nucleus than the cytoplasm.

4 Discussion

In this paper we have extended two recent spatio-temporal models of intracellular signalling pathways that were presented in Sturrock et al. (2011). These models represented the Hes1 and p53–Mdm2 pathways respectively, both of which are known to be deregulated in various cancers. We extended them by including a nuclear membrane and active transport. We accounted for the permeability of the nuclear membrane by considering its thickness and the fact that diffusion across it is slower than in the nucleus or cytoplasm, and we assumed that proteins were convected from the cytoplasm to the nucleus in order to model translocation along microtubules. The p53–Mdm2 model was also extended by accounting for Mdm2 inhibition of p53 transcriptional activity.

Experiments have shown that stimulation of the Hes1 and p53–Mdm2 pathways can cause these pathways to exhibit oscillatory dynamics, driven by a negative feedback loop in each case. Therefore we explored numerically our extended models for these pathways in the context of sustained oscillatory dynamics. We found ranges of values for the model parameters such that sustained oscillatory dynamics occurred, noting that these ranges were consistent with available experimental measurements. We also found that our model extensions acted to broaden the parameter ranges that yielded oscillations compared with the previous results of Sturrock et al. (2011). Hence oscillatory behaviour is made more robust by the inclusion of both the nuclear membrane and active transport.

Given that cell shape can influence intracellular signalling (Meyers et al. 2006; Neves et al. 2008), we investigated the effect on the dynamics of various cell geometries, finding for our extended Hes1 model that oscillatory dynamics are strongly robust to changes in the size and shape of the cell and its nucleus. Such results are consistent with other recent spatio-temporal modelling studies of intracellular signalling pathways (Terry et al. 2011; Terry and Chaplain 2011). In the interests of making accurate quantitative statements, we explored our new extended p53–Mdm2 model on a domain that was imported from an image of an osteosarcoma cell—the p53–Mdm2 pathway is known to be deregulated in osteosarcomas. We were able to make quantitative observations regarding, for example, the proportion of p53 that enters the nucleus. In particular, we saw that peaks in total nuclear concentration were 33 % the height of peaks in total cytoplasmic concentration, whereas this figure was only 8 % in the original model in Sturrock et al. (2011). Hence, although the nuclear membrane acts as a barrier to p53 nuclear localisation, active transport nevertheless increases this localisation. Our quantitative data serve
as predictions until accurate experimental data become available. We made qualitative observations too, noting that our new p53–Mdm2 model exhibited pulsatile-like dynamics in keeping with several experimental studies (Batchelor et al. 2009; Loewer et al. 2010).

Motivated by experiments involving microtubule-disrupting chemotherapeutic drugs (Jordan and Wilson 2004; Kavallaris 2010; Carbonaro et al. 2011), we considered the special case in our new models where active transport rates were set to zero. We found that this narrowed the ranges of values for model parameters such that sustained oscillatory dynamics occurred. For our p53–Mdm2 model, we found reductions in the levels of nuclear p53 and nuclear Mdm2, in qualitative agreement with experimental data in Roth et al. (2007), Giannakakou et al. (2000). We also considered the effect of proteasome inhibitor drugs in our p53–Mdm2 model by reducing protein degradation rates. This increased levels of p53 and Mdm2, especially in the nucleus, and again these results matched experimental data (Maki et al. 1996; Xirodimas et al. 2001).

From the spatial profiles for the p53–Mdm2 model, we observed that the nuclear membrane retards the nuclear entry of p53 and Mdm2, with the local concentrations of these species reaching their highest levels in or next to the nuclear membrane. Such results indicate that the nuclear pore complex is an attractive site for delivering chemotherapeutic drugs to disrupt or enhance intracellular signalling, as discussed in Gasiorowski and Dean (2003), Chahine and Pierce (2009). Our spatial profiles also suggested that microtubule-disrupting drugs may cause comparatively little protein to reach the nuclear membrane, whereas proteasome inhibitor drugs may increase protein levels both at the nuclear membrane and in the nucleus. We drew conclusions in terms of combination chemotherapy, suggesting that the effectiveness of drugs designed to act at nuclear pore complexes may be limited by microtubule-disrupting drugs but enhanced by proteasome inhibitor drugs. Computational animations of our spatio-temporal simulations closely matched the experimental results of Lahav et al. (2004) where concentration profiles of proteins in single cells were imaged utilising fluorescent fusion proteins—see the section below on Supporting Information. With the continuing advance of imaging techniques in individual cells (Kherlopian et al. 2008; Michaelet al. 2005), it will become increasingly important to model intracellular dynamics using a spatio-temporal framework.

Future work will consider extending the models further in several ways, as well as performing detailed analysis of the current models. We are currently undertaking a nonlinear analysis of the Hes1 model which has led to the study of a non-linear and nonlocal eigenvalue problem, which requires non-standard analysis and proper estimates for the corresponding Green’s function. Cells can change shape on the same timescale as oscillatory nuclear-cytoplasmic translocation of Hes1 or p53, and so we may develop a model with a moving boundary on an evolving domain. Based on cell imagery, we will consider more realistic support functions for our translation and active transport terms. We may also study the interactions between different signalling pathways. It is known, for example, that the p53–Mdm2 pathway can co-operate with and antagonise the NF-κB pathway, which is central to many stressful, inflammatory, and innate immune responses (Pommier et al. 2004; Perkins 2007). We are not aware of any spatio-temporal modelling studies of interacting pathways, though there have been temporal studies (Puszyński et al. 2009).
Our p53–Mdm2 model is based on a reduced description of the pathway, and we may explore the consequences of including more species in the model. We may also explore the effect of different chemotherapeutic drugs on the Hes1 and p53–Mdm2 pathways. There is experimental evidence that molecular movement within a cell can be “subdiffusive” or “superdiffusive” (Weiss et al. 2004; Wachsmuth et al. 2000; Caspi et al. 2000), and these are ideas we may investigate from a modelling perspective. Finally, low copy numbers of mRNA and transcription factors can cause stochastic fluctuations in the dynamics of intracellular pathways (van Zon et al. 2006; Shahrezaei and Swain 2008), and we are currently studying this.

5 Supporting Information

Animations S1 and S2. Animations of p53 concentration (S1) and Mdm2 concentration (S2) created from a simulation of the extended p53–Mdm2 model given by (19)–(26) and (44)–(45) subject to conditions (27) and (32)–(43). Parameter values are as in the second column of Table 6 and the domain on which we solve the system is the osteosarcoma cell in Fig. 13b. Time and local concentrations are shown in non-dimensional units. Our animations are qualitatively similar to the experimental results presented in the supporting online material of Lahav et al. (2004) where concentration profiles of proteins in single cells were imaged utilising fluorescent fusion proteins. See also the experimental results for p53 and Mdm2 levels in single cells in the supporting online material of Geva-Zatorsky et al. (2006).

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Appendix

A.1 Non-dimensionalisation of Hes1 Model

We summarise our non-dimensionalisation of the extended Hes1 model (described in Sect. 2.2). The original Hes1 model (described in Sect. 2.1) is non-dimensionalised in a similar way; for details, see Sturrock et al. (2011).

To non-dimensionalise the extended Hes1 model given by (1)–(4) and (15), subject to the conditions in (8)–(14), we first define re-scaled variables by dividing each variable by a reference value. Re-scaled variables are given overlines to distinguish them from variables that are not re-scaled. Thus we can write:

\[
\begin{align*}
\bar{m}_n &= \frac{m_n}{m_0}, & \bar{m}_c &= \frac{m_c}{m_0}, \\
\bar{p}_n &= \frac{p_n}{p_0}, & \bar{p}_c &= \frac{p_c}{p_0},
\end{align*}
\]

(47)

where the right-hand side of each equation is a dimensional variable divided by its reference value. From (47), we can write variables in terms of re-scaled variables.
and then substitute these expressions into (1)–(4) and (15), and into the conditions in (8)–(14). This gives a model defined in terms of re-scaled variables which has the same form as the dimensional model, but now the parameters are all non-dimensional. Denoting the non-dimensional parameters with an asterisk, they are related to dimensional parameters as follows:

\[ D^*_{ij} = \frac{\tau D_{ij}}{L^2}, \quad \alpha^*_m = \frac{\tau \alpha_m}{[m_0]}, \quad p^* = \frac{[p_0]}{\hat{p}}, \quad \mu^*_m = \tau \mu_m, \]

\[ \alpha^*_p = \frac{\tau [m_0] \alpha_p}{[p_0]}, \quad \mu^*_p = \tau \mu_p, \quad D^*_m = \frac{\tau D_m}{L^2}, \quad D^*_p = \frac{\tau D_p}{L^2}, \quad d^* = \frac{d}{L}, \quad a^* = \frac{\tau a}{L}, \quad l^* = \frac{l}{L}. \] (48)

We solve the non-dimensional model using the method described in Sect. 2.1. We simulate the model in COMSOL 3.5a, finding non-dimensional parameter values that yield oscillatory dynamics. We chose the same values as in Eq. (25) in Sturrock et al. (2011) except for those parameters which were new because of our extension to the model. These latter values were chosen as follows: \( D^*_m = D^*_{ij}/5, D^*_p = D^*_{ij}/15, d^* = 0.01, a^* = 0.03, l^* = 0.63. \)

Finally, we calculated the dimensional parameter values. To do this, we needed to estimate the reference values. Since Her1 in zebrafish and Hes1 in mice are both pathways connected with somitogenesis, we used the reference concentrations for Her1 protein and her1 mRNA in Terry et al. (2011) as our reference concentrations for Hes1 protein and hes1 mRNA. Thus, we chose \([m_0] = 1.5 \times 10^{-9} \text{ M}\) and \([p_0] = 10^{-9} \text{ M}\). We assumed a cell to be of width 30 µm. But from Figs. 2 and 4, the cell width is equal to three non-dimensional spatial units or 3\(L\)-dimensional units (using (47)). Hence we set 3\(L = 30 \mu m\), so that \(L = 10 \mu m\). The experimentally observed period of oscillations of Hes1 is approximately 2 hours (Hirata et al. 2002). Our simulations of the non-dimensionalised model gave oscillations with a period of approximately 300 non-dimensional time units or 300 \(\tau\)-dimensional units (using (47)). Hence we set 300\(\tau = 2 \text{ h} = 7200 \text{ s}\), so that \(\tau = 24 \text{ s}\). Using our references values and non-dimensional parameter values, we found dimensional parameter values from (48).

Note that we chose our reference time \(\tau = 24 \text{ s}\) based on simulations of the extended Hes1 model since this was our most realistic Hes1 model. For the original Hes1 model and for all special cases of the Hes1 model (for example, setting active transport rates to zero), we retained the reference time \(\tau = 24 \text{ s}\).

A.2 Non-dimensionalisation of p53–Mdm2 Model

We non-dimensionalised the p53–Mdm2 model defined in Sect. 3.1, and the extended p53–Mdm2 model defined in Sect. 3.2, using the technique described above for non-dimensionalising the extended Hes1 model. We give brief details for our non-dimensionalisation of the extended p53–Mdm2 model.

To non-dimensionalise the extended p53–Mdm2 model given by (19)–(26) and (44)–(45), subject to conditions (27) and (32)–(43), we define re-scaled variables...
(denoted by overlines) by dividing each variable by a reference value:

\[
\begin{align*}
[p53m_n] &= \frac{[p53m_n]}{[p53m_0]}, & [p53m_c] &= \frac{[p53m_c]}{[p53m_0]}, & [p53] &= \frac{[p53]}{[p53_0]}, \\
[p53_c] &= \frac{[p53_c]}{[p53_0]}, & [Mdm2m_n] &= \frac{[Mdm2m_n]}{[Mdm2m_0]}, & [Mdm2c] &= \frac{[Mdm2c]}{[Mdm2_0]}, \\
[Mdm2m_c] &= \frac{[Mdm2m_c]}{[Mdm2m_0]}, & [Mdm2] &= \frac{[Mdm2]}{[Mdm2_0]},
\end{align*}
\] (49)

Substituting the scaling in (49) into the extended p53–Mdm2 model gives a non-dimensionalised model with non-dimensional parameters (which we denote with asterisks) that are related to dimensional parameters as follows:

\[
\begin{align*}
D^*_{ij} &= \frac{\tau D_{ij}}{L^2}, & \xi^* &= \frac{\tau \xi}{[p53m_0]}, & \phi^* &= \tau \phi, & \beta^* &= \frac{\tau \beta [p53m_0]}{[p53_0]}, \\
\mu^* &= \tau \mu, & \nu^* &= \tau \nu, & Mdm^2 &= \frac{Mdm2}{[Mdm2_0]}, & \alpha^* &= \frac{\tau \alpha}{[Mdm2m_0]}, \\
\eta^* &= \frac{\tau \eta}{[Mdm2m_0]}, & p53^* &= \frac{p53}{[p53_0]}, & \theta^* &= \frac{[p53_0] \theta}{[Mdm2_0]}, \\
\gamma^* &= \frac{\tau \gamma [Mdm2m_0]}{[Mdm2_0]}, & \rho^* &= \tau \rho, & D^* m &= \frac{\tau D_m}{L^2}, & D^* p &= \frac{\tau D_p}{L^2}, \\
d^* &= \frac{d}{L}, & a^* &= \frac{\tau a}{L}, & l^* &= \frac{l}{L}.
\end{align*}
\] (50)

We solve the non-dimensional model using COMSOL 3.5a, finding non-dimensional parameter values that yield oscillatory dynamics. We chose the same values as in Eq. (60) in Sturrock et al. (2011) except for those parameters which were new because of our extension to the model. These latter values were chosen as follows: \(\theta^* = 1, \xi^* = 0.35, D^*_m = D^*_{ij}/5, D^*_p = D^*_ij/15, d^* = 0.01, a^* = 0.03, l^* = 0.63.\)

Finally, we calculated the dimensional parameter values. To do this, we had to estimate the reference values. As in Sturrock et al. (2011), we chose the following reference concentrations: \([p53] = 0.5 \mu M, [Mdm2] = 0.05 \mu M, [Mdm2_0] = 2 \mu M.\) In addition, we chose \([p53m_0] = 0.025 \mu M\) (in keeping with relative concentrations of mRNA and protein revealed by simulation of the non-dimensionalised model). As with the Hes1 model, we assumed a cell to be of width 30 \(\mu m\), which again leads to the reference length \(L = 10 \mu m.\) Our simulations of the non-dimensionalised model gave oscillations with a period of approximately 360 non-dimensional time units or 360 \(\tau\)-dimensional units (using (50)), and the experimentally observed period is approximately 3 hours (Monk 2003). Hence we set \(360\tau = 3 h = 10800\ s,\) so that \(\tau = 30\ s.\) The reference time \(\tau = 30\ s\) was based on simulations of the extended p53–Mdm2 model since this was our most realistic p53–Mdm2 model. For all variants of this model (for example, setting active transport rates to zero), we retained
Fig. 24 Plots showing the effect on the extended Hes1 model of varying the nuclear shape. In each row, the left plot shows the shape on which we solve, and the middle and right plots show the corresponding numerical results. Spatial units here are non-dimensional, with one non-dimensional spatial unit corresponding to 10 µm. Total concentrations for Hes1 protein are displayed in blue and for hes1 mRNA in red. Parameter values as per column 2 of Table 2

the reference time $\tau = 30$ s for ease of comparison of the numerical results. Using our references values and non-dimensional parameter values, we found dimensional parameter values from (50).

A.3 Influence of Cell Shape

As discussed in Sect. 2.2.5, we carried out simulations for the extended Hes1 model on a variety of cell geometries. Results from these simulations are presented in Figs. 24 and 25. It is clear from these figures that sustained oscillatory dynamics are strongly robust to changes in cell shape. Such robustness is reassuring since the shape of eukaryotic cells is highly variable (Baserga 2007; Pincus and Theriot 2007).

Only one of the geometries in Figs. 24 or 25 shows significant damping after the initial peaks in Hes1 protein and hes1 mRNA total concentrations. This occurs in the second row in Fig. 25, where the MTOC surrounding the nucleus is significantly increased in size. The increased size of the MTOC reduces the size of the region in...
Fig. 25 Plots showing the effect on the extended Hes1 model of varying the nucleus position (first row), the MTOC position (second row), and the cell shape (third row). In each row, the left plot shows the shape on which we solve, and the middle and right plots show the corresponding numerical results. Spatial units here are non-dimensional, with one non-dimensional spatial unit corresponding to 10 µm. Total concentrations for Hes1 protein are displayed in blue and for hes1 mRNA in red. Parameter values as per column 2 of Table 2 which active transport may occur. Hence the results in the second row in Fig. 25 are similar to those presented in Sect. 2.2.4 in which the active transport rate is set to zero.

References


