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Two different network topologies yield bistability in models of mesoderm and anterior mesendoderm specification in amphibians

L.E. Brown [a,*,1], J.R. King [b], M. Loose [c]

[a] MyCIB, School of Biosciences, University of Nottingham, Sutton Bonington LE12 5RD, UK
[b] School of Mathematical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK
[c] Centre for Genetics and Genomics, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, UK

HIGHLIGHTS

- We present models of mesendoderm specification in the urodele amphibian, the axolotl.
- *in vitro* and *in vivo* models are simulated and compared with experimental data.
- The model topology differs from that of the anuran amphibian, *Xenopus laevis*.
- Steady states representing mesoderm and anterior mesendoderm are found in both models.
- Both the axolotl and *Xenopus* topologies can account for similar qualitative data.

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ABSTRACT

Understanding the Gene Regulatory Networks (GRNs) that underlie development is a major question for systems biology. The establishment of the germ layers is amongst the earliest events of development and has been characterised in numerous model systems. The establishment of the mesoderm is best characterised in the frog *Xenopus laevis* and has been well studied both experimentally and mathematically. However, the *Xenopus* network has significant differences from that in mouse and humans, including the presence of multiple copies of two key genes in the network, Mix and Nodal. The axolotl, a urodele amphibian, provides a model with all the benefits of amphibian embryology but crucially only a single Mix and Nodal gene required for the specification of the mesoderm. Remarkably, the number of genes within the network is not the only difference. The interaction between Mix and Brachyury, two transcription factors involved in the establishment of the endoderm and mesoderm respectively, is not conserved. While Mix represses Brachyury in *Xenopus*, it activates Brachyury in axolotl. Thus, whilst the topology of the networks in the two species differs, both are able to form mesoderm and endoderm *in vivo*. Based on current knowledge of the structure of the mesendoderm GRN we develop deterministic models that describe the time evolution of transcription factors in a single axolotl cell and compare numerical simulations with previous results from *Xenopus*. The models are shown to have stable steady states corresponding to mesoderm and anterior mesendoderm, with the *in vitro* model showing how the concentration of Activin can determine cell fate, while the *in vivo* model shows that β-catenin concentration can determine cell fate. Moreover, our analysis suggests that additional components must be important in the axolotl network in the specification of the full range of tissues.

1. Introduction

Whilst far from the sole determinant of how cell types are specified, the interplay of transcription factors (TFs) and signalling molecules to form networks that regulate cell fate provides a program that underlies the development of an organism. TFs bind to promoter sites localised within target genes either to up- or to down-regulate their expression. Target genes may themselves produce TFs or signalling molecules which are secreted by the cell to activate signalling cascades and activate intracellular transducers that in turn activate target genes and so form gene regulatory networks (GRNs) driving development. During the development of an embryo from a single cell (the fertilised egg) to a fully developed multicellular adult organism, cells differentiate into increasingly specialised cell

* Corresponding author.
E-mail addresses: laura.brown@slcu.cam.ac.uk (L.E. Brown), john.king@nottingham.ac.uk (J.R. King), matt.loose@nottingham.ac.uk (M. Loose).
[1] Current address: Sainsbury Laboratory Cambridge University, Cambridge CB2 1LR, UK.

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fates. The timing and location of gene expression, as regulated by developmental GRNs, ensures that an embryo develops to form the correct body plan in the adult organism. One of the earliest events in embryo development is the formation of the three primary germ layers, mesoderm, endoderm and ectoderm (Gilbert, 2010; Slack, 1991). Each germ layer gives rise to different tissue types in the developing embryo: endoderm (the inner layer) forms the digestive system and the lungs, mesoderm (the middle layer) forms muscle, blood and connective tissue and ectoderm (the outer layer) forms the epidermis and nervous system. Collectively, the cells giving rise to both mesoderm and endoderm have been named as the mesendoderm. The specification of these cells is amongst the earliest events in the embryo and so is easily investigated experimentally.

The GRN governing the specification of mesoderm and endoderm, here termed the mesendoderm GRN (mGRN), has been studied in several species including *Caenorhabditis elegans* (Maduro, 2006), *Strongylocentrotus purpuratus* (sea urchin) (Davidson et al., 2002), *Xenopus laevis* (Loose and Patient, 2004; Koide et al., 2005) and *Ambystoma mexicanum* (Swiers et al., 2010). Notably, both *Xenopus laevis* (the frog) and *Ambystoma mexicanum* (the axolotl) are amphibians although from different orders. The frog (an anuran) and the axolotl (a urodele) differ in several significant aspects of development (Johnson et al., 2011). Remarkably these differences extend to the topology of the mGRN with significant differences in the interactions between key TFs having been identified (Swiers et al., 2010). Several mathematical models based on the *Xenopus* mGRN have been developed and analysed to provide greater understanding of how mesendoderm forms (Saka and Smith, 2007; Middleton et al., 2009). Saka and Smith (2007) show that a simple negative feedback loop can reproduce experimental observations, providing a possible mechanism for the formation of different cell types. Middleton et al. (2009) base their model on a simplified version of the *Xenopus* mGRN, representing large *Mix* and *Nodal* gene families by a single node for each gene, motivated by single copies of these genes in mammals (Guo et al., 2002; Zhou et al., 1999). The model describes the time evolution of each gene in the simplified GRN in a single cell, neglecting spatial effects. The model is able to reproduce qualitatively Activin and VegT dose response experiments, with stable steady states of the model corresponding to mesoderm and anterior mesendoderm cell fates. An important interaction for producing this behaviour is the mutual negative regulation of *Mix* and *Brachyury*. In contrast with *Xenopus*, the genome of the axolotl contains only a single *Mix* gene and two *Nodal* genes, with only one of these required for mesendoderm formation (Swiers et al., 2010). A study of the evolutionary history of *Nodal* genes suggests that ancestral species have two *Nodal* genes while higher vertebrates have lost one (Hellsten et al., 2010). Thus the expanded Mix and Nodal families in *Xenopus* are likely to be a divergent trait. Intriguingly, the relationship between *Mix* and *Brachyury* is not conserved between *Xenopus* and axolotl. The mutual negative feedback between *Mix* and *Brachyury* in *Xenopus* is key to the establishment of the mesoderm and the endoderm and so caused us to question if an alternate topology was still able to establish distinct germ layers during development. This is of particular importance as Swiers et al. (2010) show that the axolotl network, not that of *Xenopus*, is conserved with the mouse. In this paper we formulate mathematical models based on the axolotl mGRN and compare them qualitatively with the *Xenopus* mGRN, showing that the axolotl network topology can specify mesoderm and anterior mesendoderm cell fates.

2. Biological background

2.1. Mesoderm and endoderm formation in Xenopus

The GRN underlying the formation of mesoderm and endoderm in the anuran amphibian *Xenopus laevis* described in Loose and Patient (2004) and Koide et al. (2005) contains around 50 TFs and signals. Important genes within the *Xenopus* mGRN include the maternal factors VegT and β-catenin and downstream factors *Mix*.1, *Brachyury*, *Goosecoid* and the *Nodal* family. The maternal factors VegT and β-catenin provide initial positional information and initiate the expression of genes, including members of the *Nodal* gene family. Embryos depleted of VegT fail to form endoderm (Zhang et al., 1998; Kofron et al., 1999) and mesoderm (Kofron et al., 1999). The ability of VegT to induce mesoderm and endoderm is via its regulation of TGF-β (*Nodal*) signalling (Clements et al., 1999; Kofron et al., 1999). VegT can also directly activate *Mix*.1 and *Brachyury* (Loose and Patient, 2004). β-catenin is expressed in the dorsal region of the embryo following an event known as cortical rotation (Weaver and Kimelman, 2004), and by stage 9.5 its expression has spread around an equatorial ring in the prospective mesoderm (Schohl and Fagotto, 2002). Both the knockdown and the overexpression of β-catenin reveal that it regulates expression of mesodermal genes such as *Brachyury* (Schohl and Fagotto, 2003) and Nodal signalling, affecting the temporal pattern but not the overall levels of P-Smad2 activation (Lee et al., 2001). The ability of *Nodal* genes to induce mesoderm and endoderm has been investigated using Activin, an agonist of Nodal signalling. Dose response experiments (Gurdon et al., 1996, 1999; Papin and Smith, 2000; Gurdon et al., 1994; Green and Smith, 1990) show that at low concentrations of Activin a cell will become mesoderm (i.e. will express *Brachyury*). As the dose of Activin increases past a threshold value, a cell will no longer express *Brachyury* but will express *Mix*.1 (i.e. endoderm). Note that Activin is not expressed at the correct time or location to act in mesoderm and endoderm induction *in vivo* and that *Nodal-related* genes are the prime candidates for the morphogens regulating the induction of mesoderm and endoderm in *Xenopus*. FGF signals have a role in maintaining Brachyury expression in mesoderm, with a positive autoregulatory feedback loop between Brachyury and FGF (Isaacs et al., 1994). FGF also has a role in ectodermal cell fates, in particular in specifying neural tissue. Low levels of FGF in combination with an inhibition of BMP result in neural cell fates, while high levels of FGF in combination with *Nodal-related* genes result in mesodermal cell fates (Delaune et al., 2005). However, here we consider only the mGRN, which neglects factors involved in ectoderm formation, as such we consider only the positive feedback between FGF and Brachyury and neglect the role of FGF in specifying neural fates.

Cell types can be identified by the genes they express: *Brachyury* expressing cells correspond to mesoderm and *Mix*.1 expressing cells to endoderm (Lemaire, 1998). *Goosecoid* expressing cells correspond to dorsal mesoderm, which gives rise to anterior (head forming) structures (Cho et al., 1991). In this paper, we refer to cells co-expressing *Goosecoid* and *Mix*.1 as anterior mesendoderm, which forms in dorsal regions of the embryo. An important interaction in the full mGRN (Loose and Patient, 2004) is the mutual repression of *Mix*.1 and *Brachyury* (Lemaire, 1998; Latinkic and Smith, 1999) creating competition between mesoderm and endoderm which is thought to contribute to the separation of these two germ layers (Lemaire, 1998).

2.2. The axolotl mesendoderm GRN

In addition to the number of members of the *Mix* and *Nodal* gene families, there are several other differences in the topology of the axolotl and *Xenopus* mGRNs illustrated in Fig. 1(c) and discussed here. In *Xenopus*, VegT is localised to the vegetal pole of the oocyte while it is expressed uniformly throughout the oocyte in axolotl (Nath and Ellison, 2007). VegT is also found throughout the embryo in lungfish and sturgeon, suggesting that the localisation of VegT is not an ancestral vertebrate trait (Chen, 2010).