



## The control of developmental global gene expression

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**Abstract.** The complex interplay of a myriad of protein factors in embryonic development encapsulates the importance of accuracy in the control of gene expression, regulation and physical factors including cell-environment contact. *C. elegans* has an extremely similar gene interplay and hence its study has paved way a greater understanding. This review will explore cell lineage specification, mutual regulation, the consequences of mutations, and how gene regulatory networks utilise spatio-temporal triggers.

**Keywords:** *C. elegans*, Regulatory network, lineage, spatio-temporal

### 1 Introduction

#### 1.1 Lineage and Tissue segregation

Two established theories regarding the triggering of the cell differentiation from the fertilised zygote play in synchrony—beginning a complex cascade of events.

#### 1.2 Mosaic (determinate) theory

This theory involves the premise that the ovum contains morphogenic determinants or factors in the cytoplasm which then get distributed to different extents with subsequent cleavage divisions once fertilised by sperm, and hence the resultant blastomeres inherit specific compositions of mRNAs and proteins that enable them to specialise accordingly (Jeffery, 1988).

#### 1.3 Regulative (indeterminate) theory

This theory encompasses cell-to-cell induction via physical contact and this intertwines with the mosaic theory as induction would be directed according to the inherited cytoplasmic determinants (Jeffery, 1988). The regulative induction hence is dependent on the cell surroundings; if isolated from a group of cells the other cells are as a result affected and in fact compensate, for

example in the case of the formation of identical twins, through regulation (Gilbert, 2000a). As a result, the eventual variation in gene expression for differentiation is triggered by both mechanisms via the ‘commitment’ of phenotypically similar cells to a particular fate (Gilbert, 2000a).

#### 1.4 Cell Division

The initial mitotic divisions are triggered by mitosis promoting factors (MPFs) and constitute a decrease in cell size and hence cytoplasm via bypassing the growth phases and this cleavage produces an 8-cell group of totipotent blastomeres that compacts via alterations in cell-cell contact (Gilbert, 2000b). Additional divisions produce a morula of 12-15 blastomeres and with subsequent divisions a cavitation or blastocoel forms in its centre. The reduction in ovum cytoplasm with divisions is a trigger for the temporal gene expression and cleavage halts once “a new balance between nucleus and cytoplasm” is established (Gilbert, 2000b).

This review aims to incorporate what we know about gene regulatory networks to produce a logical timeline of the process and factors involved in differential gene expression for lineage specification from day 0.

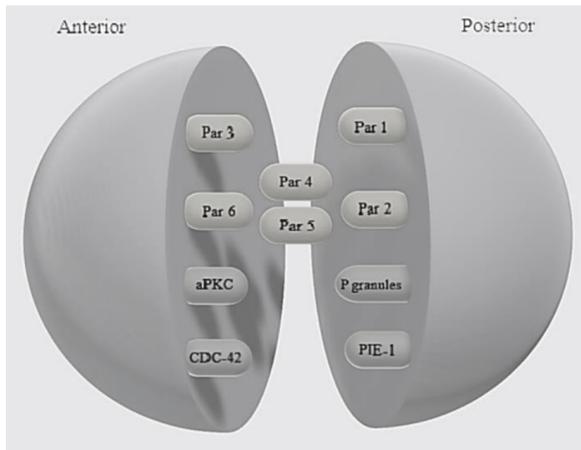
### 2 Mammalian embryo development

Asymmetric distributions of cytoplasmic molecules and proteins and the “establishment and transduction” of cell asymmetry is a ubiquitous mechanism for morphogenesis in all organisms. PAR proteins are an evolutionarily conserved trigger (Johnston et al., 2010).

#### 2.1 Blastomere polarisation

At the 8-cell stage, blastomeres undergo calcium-dependent compaction—the first true morphologic step which triggers significant steps such as initiating the differentiative division into the trophectoderm and an inner cell mass (ICM). Compaction is thought to be

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**Figure 1:** The localisations depicted by illustration of PAR proteins with associated P granules, PIE-1, aPKC and CDC-42

induced by a “minimization of the surface energy of cells” and consequently it utilises a cell-to-cell interface adhesion molecule E-cadherin – Cdh1, which partakes in the energy differences between the cells versus the cell and the external environment (Maitre et al., 2015). Oscillating contractility produces compaction through the rearrangement of actin filaments mediated by Cdh-1 (Maitre et al., 2015) and this is the ‘first step’ for blastomere polarisation (Zhu et al., 2017).

Actin rearrangement is also involved in polarising blastomere organelles and enables precursors of the trophectoderm cells (outer cells) and ICM at the 8-cell stage to form 16 cells: providing the first means of directing cell fate (Humiecka et al., 2017). Cytoplasmic components that are localised in the apical portion include PAR proteins which omits actin and produces a “mature apical cap” (Zhu et al., 2017).

## 2.2 PAR proteins

PAR3 and PAR6 interact with atypical protein kinase C (aPKC) via their association with actin (Zhu et al., 2017). Actomyosin accumulates apically because before fertilisation, the sperm pronuclei enters and hence establishes the ‘posterior’ pole via inducing the “local loss of the uniform NMY-2 network” (via downregulation of RHO-1 proteins and accumulation of PPK-1) that was previously ubiquitous around the whole ovum cortex (Johnston et al., 2010; Nance et al., 2011). RHO-1 downregulation alongside areas of RHO-1 upregulation controls the distribution of contractility, directing actomyosin fibre contraction away from the posterior pole hence constituents are directed to and accumulate in the apical pole (Johnston et al., 2010). Experiments highlight the effects of PAR protein loss, where maternal par gene mutant screens resulted in the “first embryonic cleavage to be symmetric” (Nance et al., 2011).

As illustrated in figure 1 apical localisation of PAR3, PAR6 and aPKC contrasts to PAR1 and PAR2 localisation posteriorly whereas PAR4 and PAR5 are unaffected in its distribution due to the anteriorly localised PAR proteins which interact with the other PAR proteins via “inhibitory interactions” (Nance et al., 2011). There is differential accumulation due to a variety of mechanisms such as PAR-3 association with NMY-2 (depleted posteriorly) as shown by experiments utilising dysfunctional F-actin and thus leading to lack of PAR-3 apically (Nance et al., 2011). However, asymmetry of both actomyosin and the PAR proteins is not definitively or strictly linked since experimental induction of actomyosin asymmetry early (in a 4-cell embryo of *C. elegans*) does not independently lead to PAR protein apical localisation and hence there must be other components interacting with actomyosin in order to ‘carry’ PAR proteins apically i.e. intermediate factors that supposedly physically links the two (Zhu et al., 2017).

PAR proteins are kinases which once directed, initiate a cascade of effects reinforcing cell asymmetry via phosphorylation, however “only a few direct targets for these kinases are currently known” (Johnston et al., 2010). Asymmetry of PAR proteins enables the subsequent “asymmetries in mRNA and protein” mainly via intermediates MEX-5 and MEX-6 and therefore affect the localisation of developmental lineage determinants (Nance et al., 2011; Tenlen et al., 2008).

Ezrin, actin, PAR3/6 and aPKC all contribute to the apical cap. Phosphorylated ezrin becomes localised apically after compaction uses actin to aid the formation of the apical microtubule cap by linking actin to the cell membrane (Humiecka et al., 2017).

## 3 Translational control

Spatially and temporally controlled translation of maternal mRNAs via maternal RNA binding proteins (RBPs) enables cell specification (Oldenbroek et al., 2013). The maternal RBPs for the maternal mRNA *zif-1* include “OMA-1, OMA-2, POS-1, SPN-4, MEX-3, MEX-5 and MEX-6” where different RBPs have different roles; such as MEX-3 mediated inhibition of translation in 1 or 2-cell embryos (Oldenbroek et al., 2013).

### 3.1 MEX-5 MEX-6

MEX-5 is a zinc finger protein that becomes apically localised as a result of PAR proteins—from being distributed evenly in the ovum, it is dependent on PAR 1 proteins that have become localised posteriorly, and also PAR 4 proteins which phosphorylate the C terminal Ser 458 facilitating mobility and therefore localisation to the apically localised actomyosin filaments (Daniels et al., 2010; Nance et al., 2011; Tenlen et al., 2008). MEX 5 is the first “somatic determinant” defining its lineage progression and experiments have indicated that it ant-

agonises the expression of any germline proteins where it localises anteriorly (Daniels et al., 2010). MEX-5 binds to the RNAs *glp-1* and *nos-2* leading to the inhibition of expression of proteins “SKN-1, PIE-1, NEX-1, POS-1 and PAL-1” alongside expression of the proteins “GLP-1 and MEX-3” and specific destruction of some zinc finger proteins (Pagano et al., 2007).

MEX-6 is a very similar zinc finger protein localised apically, working concurrently with MEX-5 to produce localisation of germline proteins at the posterior pole (Johnston et al., 2010).

### 3.2 PIE-1

PIE-1 is (mainly) a nuclear protein and the first “germline determinant” also localised by PAR proteins in the nucleus posteriorly, where it inhibits gene expression in germline-fated cells (Daniels et al., 2010). The dependency between PIE-1 and PAR protein localisation was demonstrated by *par* mutants which caused a lack of MEX5/6 and PIE-1 polarisation yet PIE-1 mutants did not affect the other two proteins, i.e localisation of PIE-1 (and MEX5/6) is a consequence of PAR protein asymmetry, however, MEX5/6 was also identified to be affecting the polarisation of PAR proteins and hence provides a mutual interaction for asymmetry (Johnston et al., 2010). In addition, some PIE-1 proteins are bound to “large RNA/protein granules” found posteriorly known as P granules, which are directed posteriorly through MEX5/6 mediated antagonism anteriorly (MEX5/6 mutants led to the lack of posterior localisation of P granules and hence also of PIE-1 indirectly) (Johnston et al., 2010). MEX-5/6 localising anteriorly also antagonises other germline lineage proteins such as POS-1 and MEX-1 which like PIE-1 distributes posteriorly (Daniels et al., 2010). PIE-1 prevents the action of transcription factors and hence the transcription of mRNAs leading to somatic lineage such as SKN-1, protecting totipotency of germline lineage blastomeres (Tenlen et al., 2008).

### 3.3 Other germline determinants: POS-1 and MEX-1

POS-1 and MEX-1 are also germline lineage proteins but are located in the cytoplasm and promotes the translation of mRNAs of germline-fated daughter cells (Daniels et al., 2010). POS-1 mutants alone cause blastomeres to switch to a somatic-fated cell, yet abnormalities differ from MEX-1 and PIE-1 mutants, indicating “distinct roles in the specification of germline blastomeres” (Tabara et al., 1999). Furthermore, MEX-1 and POS-1 proteins also associate with P granules (Tabara et al., 1999).

### 3.4 Other somatic line determinants: PLK-1 and CDC-25

These provide an insight into the intertwining of cell cycle progression and asymmetry since MEX-5/6 binds to PLK-1 and hence aids its anterior apical cytoplasmic localisation and so is present in somatic (AB), versus germline (P), where it triggers “earlier mitotic entry” (Noatynska et al., 2013). To prevent MEX-5/6 binding (directly) to PLK-1 before it itself has been apically localised, it is inactive until it is phosphorylated — at the end of meiosis II, Cyclin-Dependent Kinase 1 (CDK-1) phosphorylates MBK-2, which in turn phosphorylates MEX-5 (Noatynska et al., 2013).

CDC-25, a cell division cycle kinase (cyclin dependent) accumulates anteriorly in the nucleus due to PLK-1 to “promote differences” in the timing of cell cycles (Noatynska et al., 2013). An increased concentration of PLK-1 anteriorly (cytoplasm) results in an increase in localised “nuclear CDC-25 in the AB cell compared to the P1 cell” (Johnston et al., 2010).

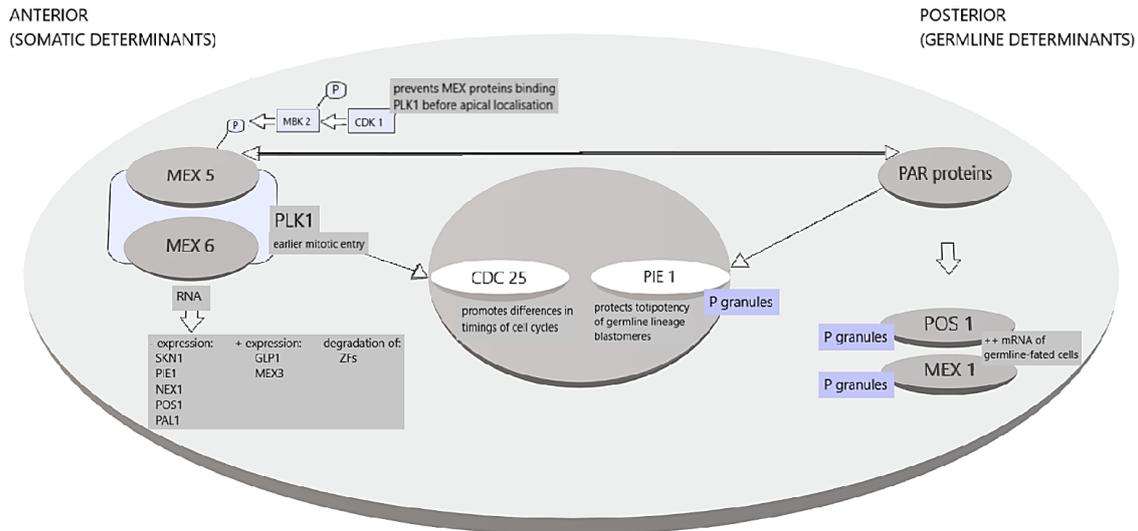
## 4 Cell Division for Differentiation

Germline blastomeres are totipotent and after a few divisions from the 1-cell stage, a point is reached where further divisions only produce germline daughter cells; possibly due to temporal differentiation differences since there is a link between the inherited ovum mRNAs and posteriorly localised factors with totipotent characteristics in germline blastomeres (Tabara et al., 1999). Moreover, gene expression varies between germline and somatic blastomeres since at the 4-cell stage when gene transcription begins, “mRNA transcripts are detected only in the somatic blastomeres” yet inhibited in germline blastomeres, which are therefore unaffected by evenly distributed maternal transcription factors, which subsequently define gene expression in somatic lineage blastomeres after the 4-cell stage (Tabara et al., 1999).

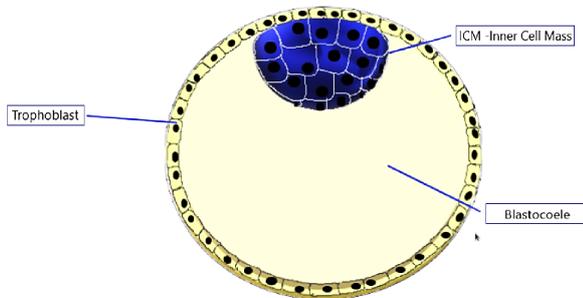
## 5 ICM and Trophectoderm development

After 1–2 weeks the morula forms a blastocyst consisting of outer specified cells contributing to the trophectoderm and an inner totipotent group of cells termed the inner cell mass surrounded mostly by a fluid cavity termed the blastocoel.

Cells are ‘committed’ to either by differentiative division where the apical (polar) cell contributes to trophectoderm and the basal (apolar) cell to the ICM; similarly to stem cells, whereas conservative division enables both daughter cells to be part of the same cell population as it distributes cell constituents equally (Humiecka et al., 2016). Differentiative cell division involves differentiative contractility if “differences in surface contractility... exceeds a predictable threshold” cells become in-



**Figure 2:** A summary of the effects of apical localisation of MEX 5 and MEX 6 somatic determinants (AB), alongside posteriorly localised PAR proteins and P granules as germline determinants that protect blastomere totipotency (P)



**Figure 3:** The morula at 1–2 weeks initially consists of a totipotent mass of cells—the inner cell mass—derived from apolar cells, and the outer trophoctoderm consisting of trophoblasts derived from apical cells. The blastocoele is a fluid filled cavity or ‘blastocyst cavity’

ternalised for this first lineage specification (Humiečka et al., 2016).

### 5.1 Trophectoderm

Cdx2, a caudal-related homeobox transcription factor, is the initial transcription factor only expressed by the trophoctoderm and hence enables implantation where abolition in experiments utilising RNAi of Cdx-2 mRNA results in the “failure of embryos to... implant” (Wu et al., 2010). Cdx2 also activates the progressive expression of Hox genes to establish an anterior-posterior axis (Wu et al., 2010). The Cdx-2 target Eomesodermin is a transcription factor that enables the specialisation of cells becoming part of the polar or mural trophoctoderm and another transcription factor Elf5 enables specification of polar trophoctoderm cells determined to be extra-embryonic ectoderm (EEE) or cells producing the ectoplacental cone (Degrelle et al., 2005).

The trophoctoderm contains trophoblast stem cells—TS cells, maintained by internal trophoblastic cells (the ‘polar trophoctoderm’, versus the external ‘mural trophoctoderm’) which are closer to the ICM and hence receiving of the ICM paracrine factor FGF4 (Marikawa et al., 2009). FGF4 stimulates the trophoctoderm to form the EEE which in turn produces BMP4 which aids in the differentiation of the ICM by directing its patterning while the EEE itself eventually develops to form the embryonic side of the placenta—the chorion (Marikawa et al., 2009).

Cdx-2 null embryos have highlighted the role of Cdx-2 in late blastomeres where the formed blastocoele starts to fail due to a possible increase in apoptotic activity alongside the failure of “epithelial integrity” of external trophoctoderm, however there is still an increase in Oct4 expression, a protein specifically expressed in wild-type ICM cells (Marikawa et al., 2009). Cdx-2 has been identified to compartmentalise the expression and hence effects of Oct-4 to the ICM; consequently the specification of first lineages arises from intertwining mechanistic effects of stimulatory and inhibitory regulation in contrast to a linear sequence of sequential gene expression, providing a means of mutual antagonism and hence restricted differential specialisation (Marikawa et al., 2009).

### 5.2 Interaction between trophoctoderm and ICM (Yap / HIPPO pathway)

It is apparent that Oct4 protects the totipotent and pluripotent cells of the ICM from differentiating into trophoctoderm and its two major targets has been identified as the FGF-4 and Nanog genes (Marikawa et al., 2009). Oct-4 expression in the ICM essentially provides

the polar trophectoderm with its co-expressed signal, FGF-4 to enable its stability and development and additionally through targeting the Nanog gene, it preserves the plasticity of embryonic stem cells by inhibiting its differentiation into primitive endoderm and the pluripotency of the ICM (Marikawa et al., 2009).

Tead4 is a transcription factor expressed in both the ICM and the trophectoderm at the 8-cell stage and studies using Tead-4 null embryos displayed normal ICM progression yet failures in trophectoderm specification and development such as the lack of blastocoel formation (in comparison to the formation then failure of the blastocoel with Cdx-2 null embryos) and hence it can be deciphered that Tead-4 may act on the gene encoding Cdx-2 as well as other targets that caused a greater subsequent effect than that of just Cdx-2 null embryos alone (Kaneko et al., 2013; Marikawa et al., 2009). Since the gene for Tead-4 is “genetically upstream” relative to the gene encoding Cdx-2, this indicates that it may not be required for the differentiation of stem cells into the trophectoderm lineage (Nishioka et al., 2009). On the other hand, it was also decided that the maintained potential of TS cells was not “fully substituted” by Cdx-2 alone in Tead-4 null embryos and subsequently, Tead-4 is more important than Cdx-2 in determining the trophectoderm lineage (Nishioka et al., 2009). Furthermore, even though Tead-4 was “genetically upstream”, Cdx-2 null embryos failed to produce TS cells and hence it is also essential for cells of the trophoblastic lineage but in a way in which “Tead-4 promotes trophoblast fate through both Cdx-2-dependent and independent pathways... while Cdx-2 is a major mediator of Tead-4 dependent changes in trophoblast gene expression” (Nishioka et al., 2009).

The mechanism in which Tead-4 null embryos lead to blastocoel failure was linked to an increase in ROS production since the development of the blastocoel involves “increased oxidative phosphorylation” and hence ROS (Kaneko et al., 2013). It is worth noting that Tead-4 is not vital for the lineage determination itself of trophoblastic cells since “once the trophectoderm was specified, Tead-4 was not essential for either proliferation or differentiation of trophoblast cells in culture”, but that Tead-4 is vital in “maintaining energy homeostasis” during early embryonic development prior to complete invasion of the mural trophectoderm into the uterus (Kaneko et al., 2013).

Tead-4 is not localised to the cytoplasm, but its selective action relies on the transcriptional co-activator YAP1 which is found specifically in the nuclei trophectoderm cells (Nishioka et al., 2009). The Hippo pathway is a cascade that directs the distribution of YAP1 in a way that enables its binding transcription factor Tead-4 to be functional in the trophoblastic lineage cell popu-

lations.

The differential activation (in contrast to the usual mechanism of differential expression) of Tead-4 determines trophoblastic lineage since it is found distributed evenly throughout the blastomeres. YAP1 found in the nuclei of trophectoderm cells from the 8-cell stage is localised through the action of a Hippo signalling pathway factor called Lats2 which is a kinase that phosphorylates and removes YAP1 from the nuclei of cells in the ICM and hence prevents the transcription of Tead-4 in the ICM (Sozen et al., 2014). Yap1 defective embryos still developed functional trophectoderm and so although Tead-4 functions with and without Cdx-2, there must be another way of transcribing Tead-4 initially and the trigger was found to be similar in structure to YAP1 — the cofactor WWTR1 (Nishioka et al., 2009; Sozen et al., 2014).

Similarly to compaction triggering the first steps towards cell specification, cell-cell contacts also play a role in Hippo signalling to be active in the ICM and not the trophectoderm in order to localise Tead-4 appropriately and hence trophectoderm commitment in these outer cells (Sozen et al., 2014). This is achieved by the extensive cell-cell contacts between cells of the ICM and was demonstrated by an experiment looking at the effects of a lack of cell where “differentiation towards TE, possibly as a result of the inactive Hippo pathway, decreased cell polarity and increased levels of Cdx2” (Sozen et al., 2014). The Hippo pathway is specific to the internal, high cell-cell contacted cells of the ICM because of the specification mechanisms utilising cell positioning and cell asymmetry—demonstrated when the apical cap complex was targeted in early embryos, YAP-1 was reduced in the outer (polar) cells (Hirate et al., 2013).

Asymmetric distribution of proteins Amot and Amotl2 have been highlighted as essential for ICM linked Hippo pathway activity by binding to actin in adherens junctions between these cells and through the high cell-cell contact, activating the Hippo signalling pathway in these non-polar cells, meanwhile in outer cells, as Amot is apically localised via the PAR-aPKC complex, because the basal adherens junctions are not devoid of Amot they fail to activate Lats2 and therefore YAP1 / WWTR1 and therefore Tead-4 (Hirate et al., 2013).

## 6 ICM second lineage segregation

Prior to the implantation of the embryo there is further differentiation of the ICM, approximately 24 hours after the formation of the trophectoderm, into a pluripotent epiblast which eventually forms the embryo itself, and an endoderm (primitive endoderm – PE) which forms extra-embryonic endoderm (Humiecka et al., 2016). Secondary lineage commitment is under the influence of

various “lineage-specific transcription factors” that are favoured in a regulatory manner (Sozen et al., 2014).

Mutual antagonism between the two lineages within the ICM — particularly the establishing markers Gata 6 in the primitive endoderm with Nanog in the epiblast, is partially maintained by FGF4 and FGFR2 which are initially ubiquitous in the ICM until the 64-cell stage when FGFR2 becomes upregulated in the primitive endoderm lineage cells and FGF4 in the epiblast lineage cells (Sozen et al., 2014). FGF4 is also upregulated in the primitive endoderm and it potentiates the action of FGFR2 in maintaining the expression of Gata 4/6 and Sox-17 thus there is potentiated antagonism of Nanog via the increase in Gata 6 concentration (Sozen et al., 2014). Although FGF4 is also upregulated in the epiblast lineage cells and so both lineages are dependent on FGF4, FGFR2 is downregulated and so are its subsequent effects (Sozen et al., 2014).

Although there are two lineages forming in the ICM, they are scattered, and this heterogeneity is a result of different transcripts since signalling rather than cell position triggers specification. A ‘cell sorting model’ was proposed which encompasses organisation via “waves of asymmetric divisions” that results in relative amounts of each lineage type to change—that of primitive endoderm increases and vice versa for that of the epiblast, and how during the formation of the blastocoel, movement of cells towards the outside or inside can occur but is regulated by the transcription markers on their cell surface (Artus et al., 2010; Sozen et al., 2014). The presence of proteins such as DAB2 and ECM LAMC1 have also been found to play a role in cell sorting Artus et al. (2010). Cells with transcription markers indicating epiblastic lineage i.e. expression of a larger amount of Gata-6, tend to have less cell-cell adhesion and so tend to move inwards and those expressing large amounts of Nanog tend to have higher cell-cell adhesion and move externally (Sozen et al., 2014).

## 7 Conclusion

Calcium-dependent compaction, as the first morphological change, is in essence the most important factor controlling early developmental gene expression since it enables the subsequent chain of events establishing networks of gene expression through changes in cell surface energy; which results in the formation of an apical cap and therefore the polarisation of blastomeres. This apical polarisation complex is the means by which cytoplasmic determinants, maternal mRNAs and proteins are differentially localised and hence subsequently cause compartmentalised gene expression for the progression into specific developmental lineages.

Recurring methods of control are evident throughout early development — such as cell-cell contact being a

regulator initially in compaction, but it is also a method involved in Hippo signalling and again later in cell sorting after transcriptional heterogeneity.

## Declaration of Interest

There are no conflicts of interest.

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