factors) is insufficient to specify homogeneous DE (quantified, by Chetty et al., 2013). BMP, FGF, VEGF and Wnt have also been employed together with TGFβ signals to generate DE (Cheng etal., 2012; Goldman et al., 2013; Green et al., 2011; Kroon et al., 2008; Nostro et al., 2011; Touboul et al., 2010). However, these morphogens are also implicated in mesoderm commitment (Davis et al. , 2008; Gertow et al., 2013) and their precise involvement in DE induction remains to be clarified.

[0006] There is currently no coherent understanding of the signaling logic underlying multiple steps of induction and patterning of the germ layers and differentiation into the various cell lineages.

[0007] It is an aim of the present invention to elucidate the underlying signaling logic of stem cell induction and differentiation in order to unilaterally drive stem cells to a single cell fate with minimal extraneous lineages,

SUMMARY

[0007a] In one aspect, provided herein is a method of differentiating pluripotent stem cells into definitive endoderm (DE) lineage comprising

i) contacting said pluripotent stem cells with:

a. 100ng/ml to 10µg/ml of one or more activators of TGFβ/Nodal signaling; and

b. about 2µM of one or more activators of Wnt signaling, wherein the one or more activators of Wnt signaling are CHIR99021 or Wnt3a

to produce cells of the anterior primitive streak (APS) lineage in 24 hours; and

ii) contacting said APS cells with:

a. 100ng/ml to 10µg/ml of one or more activators of TGFβ/Nodal signaling; and

b. about 250nM of one or more inhibitors of BMP signaling or 1µM to 4µM of one or more inhibitors of Wnt signaling for 24 hours to 96 hours

to produce cells of the DE lineage.

[0007b] In another aspect, provided herein is a cell culture medium when used in the method as described herein for differentiating a pluripotent stem cell into APS lineage comprising: a matrix component, one or more activators of TGFβ/Nodal signaling, and one or more activators of Wnt/β-catenin signaling.

[0007c] In another aspect, provided herein is a cell culture medium when used in the method as described herein for differentiating an APS cell into DE lineages comprising: one or more activators of TGFβ/Nodal signaling, one or more inhibitors of BMP signaling or one or more inhibitors of Wnt signaling.

[0007d] In another aspect, provided herein is a kit when used in the method as described herein, comprising one or more containers of cell culture medium as described herein, together with instructions for use.

[0008] According to one example there is provided a method of differentiating stem cells into one or more cell lineages comprising contacting said cells with one or more activators of TGFβ/Nodal signaling and one or more activators of Wnt signaling.

[0009] According to another example there is provided a method of differentiating anterior primitive streak cells into cells of definitive endoderm (DE) lineage, by contacting said cells of the anterior primitive streak lineage with one or more activators of TGFβ/Nodal signaling, and one or more inhibitors of BMP signaling, or one or more inhibitors of Wnt signaling.

[0010] According to another example there is provided a method of differentiating cells of the DE. into cells of the AFG, by contacting said DE cells with a TGFP inhibitor and a BMP inhibitor.

Claims

1. A method of differentiating pluripotent stem cells into definitive endoderm (DE) lineage comprising
   i) contacting said pluripotent stem cells with:
   a. 100ng/ml to 10µg/ml of one or more activators of TGFβ/Nodal signaling; and
   b. about 2µM of one or more activators of Wnt signaling, wherein the one or more activators of Wnt signaling are CHIR99021 or Wnt3a
   to produce cells of the anterior primitive streak (APS) lineage in 24 hours; and
   ii) contacting said APS cells with:
   a. 100ng/ml to 10µg/ml of one or more activators of TGFβ/Nodal signaling; and
   b. about 250nM of one or more inhibitors of BMP signaling or 1µM to 4µM of one or more inhibitors of Wnt signaling for 24 hours to 96 hours
   to produce cells of the DE lineage.

2. The method as claimed in claim 1, wherein the one or more activators of TGFβ/Nodal signaling is Activin A, TGFβ1, TGFβ2 or Nodal.

3. The method as claimed in any one of the preceding claims, wherein the one or more inhibitors of BMP signaling is DM3189/LDN-193189, noggin, chordin, or dorsomorphin or DMH1.

4. The method as claimed in any one of the preceding claims, wherein the pluripotent stem cells are contacted with Activin A, and CHIR99021.

5. The method of claim 4, wherein the pluripotent stem cells are contacted with 100ng/ml to 10µg/ml of Activin A and about 2µM of CHIR99021.

6. The method of claim 5, wherein the pluripotent stem cells are contacted with about 100ng/ml of Activin A and about 2µM of CHIR99021.

7. The method of any one of the preceding claims, wherein the cells of the anterior primitive streak lineage comprise elevated gene expression of anterior streak or pan-streak markers and decreased expression of posterior streak markers relative to undifferentiated cells.

8. The method of any one of the preceding claims, wherein the APS cells are contacted with 100ng/ml to 10µg/ml of Activin A and about 250nM of LDN-193189.

9. The method of claim 8, wherein the APS cells are contacted with about 100ng/ml of Activin A and about 250nM of LDN-193189.

10. The method as claimed in any one of the preceding claims, wherein the one or more inhibitors of Wnt signaling is Iwp2, Dkk1, C-59, or Iwr-1 or XAV-939.

11. The method of claim 10, wherein the APS cells are contacted with 100ng/ml to 10µg/ml of Activin A and 4µM of Iwp2 or 1µM C-59.

12. The method of claim 11, wherein the APS cells are contacted with about 100ng/ml of Activin A and about 4μM of Iwp2 or about 1 μM of C-59.

13. The method of any one of the preceding claims, wherein the cells of the definitive endoderm lineage comprise elevated endoderm gene expression and decreased pluripotency gene expression relative to undifferentiated cells.

14. The method of any one of the preceding claims, wherein the cells of the definitive endoderm lineage comprise significantly reduced mesoderm gene expression.

15. A cell culture medium when used in the method of any one of claims 1 to 14 for differentiating a pluripotent stem cell into APS lineage comprising: a matrix component, one or more activators of TGFβ/Nodal signaling, and one or more activators of Wnt/β-catenin signaling.

16. A cell culture medium when used in the method of any one of claims 1 to 14 for differentiating an APS cell into DE lineages comprising: one or more activators of TGFβ/Nodal signaling, and one or more inhibitors of BMP signaling or one or more inhibitors of Wnt signaling.

17. A kit when used in the method of any one of claims 1 to 14, comprising one or more containers of cell culture medium as claimed in any one of claims 15 to 16, together with instructions for use.