Seminars in Cell and Developmental Biology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Seminars in Cell and Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Peeking under the hood of early embryogenesis: Using tools and synthetic biology to understand native control systems and sculpt tissues

Wenchao Qian [a,c], Matthew C. Good [a,b,c,d,*]

[a] Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA 19104, USA
[c] Cell and Molecular Biology Graduate Program, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
[d] Lead Contact, USA

ARTICLE INFO

Keywords:
Embryogenesis
Synthetic developmental biology
Blastula
Thermal regulation
Embryo temperature controller
Optogenetics
Optochemistry
Self-organization
Micropatterning
Confinement
Encapsulation
Templated development

ABSTRACT

Early embryogenesis requires rapid division of pluripotent blastomeres, regulated genome activation, precise spatiotemporal signaling to pattern cell fate, and morphogenesis to shape primitive tissue architectures. The complexity of this process has inspired researchers to move beyond simple genetic perturbation into engineered devices and synthetic biology tools to permit temporal and spatial manipulation of the control systems guiding development. By precise alteration of embryo organization, it is now possible to advance beyond basic analytical strategies and directly test the sufficiency of models for developmental regulation. Separately, advances in micropatterning and embryoid culture have facilitated the bottom-up construction of complex embryo tissues allowing *ex vivo* systems to recapitulate even later stages of development. Embryos fertilized and grown *ex vivo* offer an excellent opportunity to exogenously perturb fundamental pathways governing embryogenesis. Here we review the technologies developed to thermally modulate the embryo cell cycle, and optically regulate morphogen and signaling pathways in space and time, specifically in the blastula embryo. Additionally, we highlight recent advances in cell patterning in two and three dimensions that have helped reveal the self-organizing properties and gene regulatory networks guiding early embryo organization.

1. Section I. Tools for spatial regulation of embryo division, blastula organization, cell signaling, and patterning

Early embryo development requires careful orchestration of signaling gradients and cell fate determination to effectively proceed through process of morphogenesis. Genetic manipulation represents one strategy to analyze the gain or loss of protein activities during the process of tissue construction. However, given the complexity of the embryonic tissue, it has become clear that we require sophisticated approaches to modulate activities at precise times and in distinct regions of space. Synthetic biology methods have been developed to both sense embryonic signals and to manipulate pathway activities, identifying critical periods and allowing researchers to perform sufficiency tests. In this first section we review devices and molecular tools used to manipulate the spatial organization of the embryo through perturbations to blastomere divisions, alteration of signaling pathway flux, and patterning of morphogen gradients. These approaches allow precise spatiotemporal control over embryonic activities, providing insights on embryo control systems that would be difficult to deduced from simple genetic manipulation alone.

1.1. I.A. Thermal control of embryonic divisions

Oocytes represent a specialized cell type of exceptionally large size. Upon fertilization, the egg undergoes rapid divisions in the absence of cell growth to generate the blastula embryos containing hundreds to thousands of cells called blastomeres. This ball of pluripotent cells is then sculpted in the process of morphogenesis including early tissue organization during gastrulation [1]. In some organisms, including amphibian embryos, the blastula cleavages are not spatially uniform. Unequal cell cycle periods and asymmetric divisions generate an anisotropic ordering of blastomeres: more divisions and smaller cells on the animal pole (AP) and fewer divisions and larger cells toward the vegetal pole (VP) [2–4].

To better understand the control systems governing early cleavages and the robustness of patterning formation, various groups have

* Correspondence to: 1151 Biomedical Research Building, 421 Curie Blvd, Philadelphia, PA 19104, USA.
E-mail address: mattgood@pennmedicine.upenn.edu (M.C. Good).

https://doi.org/10.1016/j.semcdb.2022.04.016
Received 27 February 2022; Accepted 13 April 2022
1084-9521/

Please cite this article as: Wenchao Qian, Matthew C. Good, *Seminars in Cell and Developmental Biology*, https://doi.org/10.1016/j.semcdb.2022.04.016

developed devices to generate temperature gradients in blastulae, thereby uncoupling cell cycle periods spatially. Early efforts to manipulate the spatial organization of the embryo utilized thermal gradients or steps within agarose blocks or within microfluidics devices to create developmental asynchrony spatially within the tissue [5,6]. For example, the rate of nuclear division was manipulated along the anterior-posterior axis in syncytial *Drosophila* embryos, using a temperature step 17–27 °C (Fig. 1A). However, although desynchronization could affect the timing of visible stripe patterning in early development, the embryos compensated and did not experience major defects in later development [5]. Even with temperature inversion, larvae developed normally with the correct number and pattern of segments.

A conceptually similar thermal manipulation strategy was used to interrogate whether blastomere cleavages are coupled to a global division wave upon fertilization of eggs from a vertebrate model, *Xenopus laevis*. Along with other amphibian embryos, *Xenopus* embryos, contain an animal-to-vegetal cell size gradient in the blastula stage generated by asymmetry division and unequal cell cycle period along the animal-vegetal axis. To decouple cell division timing in the embryo, the authors constructed an embryo temperature controller consisting of two aluminum blocks attached to peltier thermocouples [4] (Fig. 1B). Interestingly, almost a hundred years ago, researchers also applied temperature gradients, albeit less sophisticated, to amphibian embryos and characterized developmental outcomes [7]. In this more modern embryo temperature controller strategy, the authors sped up or slowed down the cell division period on opposite sides of the embryos, including along the animal-vegetal pole axis or side to side along the equator. In these cleavage stage embryos, cells divide without growing and thus the number of cell divisions dictates blastomere size. Further, because achieving a threshold blastomere size is coupled to cell cycle elongation and genome activation [2], alteration of the spatial gradient of blastomere sizes offers the possibility to alter the timing and spatial onset of zygotic transcription and potentially germ layer specification. With this modern tool in hand, future studies should be able to address the evolutionary role of cell size gradient by flattening or inverting it and analyzing the impacts on subsequent development. It is worth noting that these experiments may yield findings that are quite different than those in *Drosophila*. Instead of imposing an artificial nuclear cycle gradient on embryos that are normally spatially homogenous, these studies would remove or alter a pre-existing gradient that appears

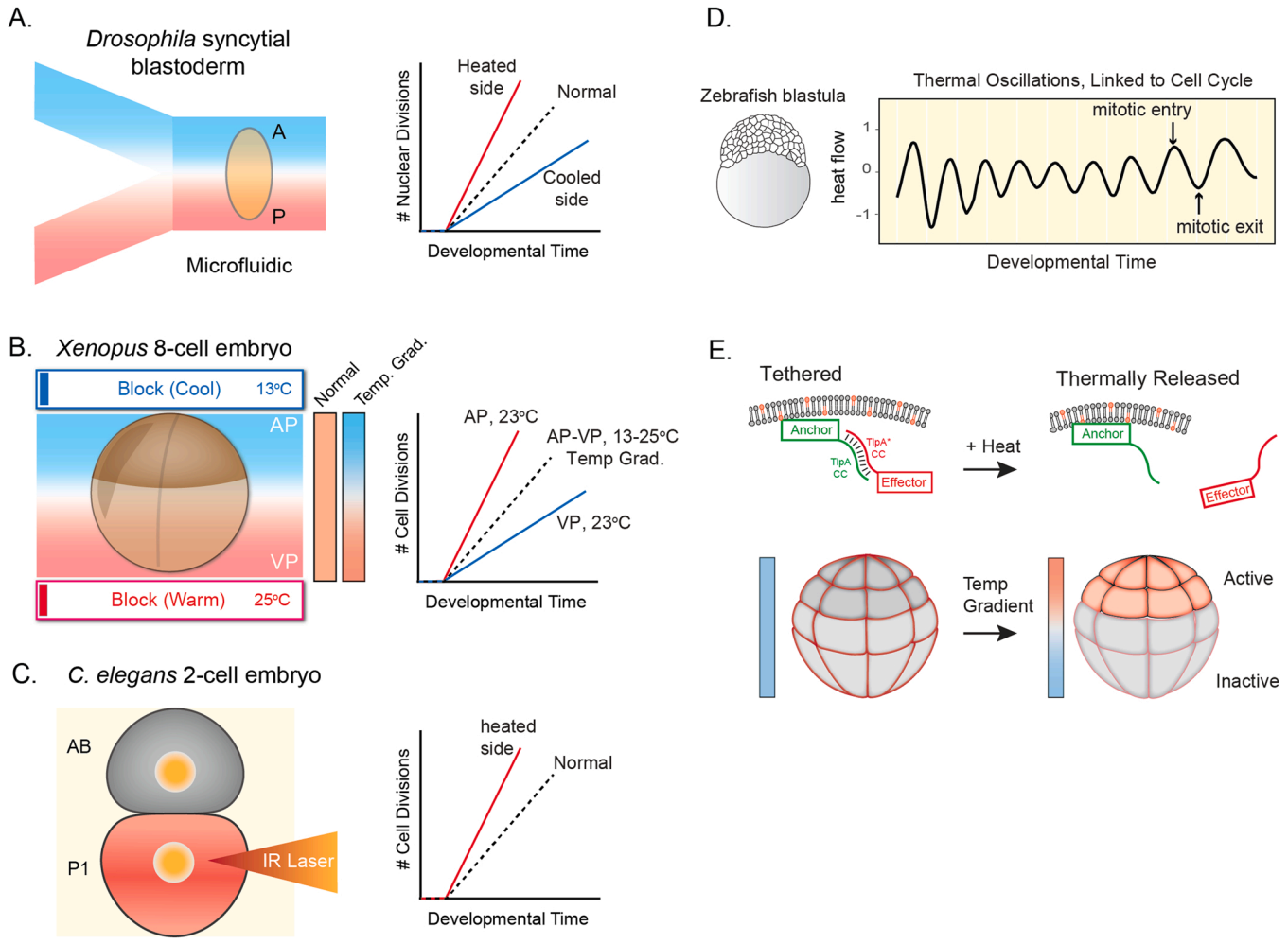

Fig. 1. Thermal approaches to manipulate the growth rate and spatial organization of embryos. (A) A microfluidic device with two temperature streams was used to create a thermocline along the anterior-posterior axis of syncytial *Drosophila* embryos, creating a spatial discoordination of nuclear division numbers. The slope of graph shows the division rates on two sides of the embryo. (B) Embryo temperature controller used to apply a thermal gradient to *Xenopus laevis* blastula, starting from 8-cell stage; reversing the natural animal-to-vegetal blastomere cell size gradient. Graph shows division rate of different regions under no normal room temperature versus in temperature controller device. The difference in cell division numbers of the animal pole (AP) and vegetal pole (VP) is diminished upon thermally constrained cell division rates. (C) IR laser is used to locally heat either half of two-cell *C. elegans* embryos. In the configuration shown, local heating speeds up the cell division of P1 cells but not AB cells. (D) In the developing zebrafish blastula, the cell cycle is correlated to heat flow with the surrounding medium. It is highest upon mitotic entry and minimum at mitotic exit. (E) Conceptual experiment: thermal release of effector protein could be coupled to temperature controller devices or ultrasound sources to spatially heat embryonic tissue, causing a localized release of an effector, such as a transcription factor or activated kinase.

conserved in blastulae of many amphibian species.

Recently, novel approaches have been developed that leverage both localized heating for temperature control and nanoscale thermometry. Thermally responsive fluorophores have been established [8], however, their implementation in live embryonic tissues is challenging and the sensors have limited dynamic range. In recent work, nanodiamond temperature sensors were used to measure temperature in the AB and P1 cells of *C. elegans* 2-cell embryos [9], and also in adult tissues [10]. In the early embryo, the authors characterized the effects of local infrared laser illumination which produces a temperature gradient across the tissue (Fig. 1C). They found that the embryo development was very robust, even after inversion of cell-division times, as most of the heated embryos hatched and grew into adulthood.

Intriguingly, embryos experience natural temporal cycles of heating and cooling related to the cell division cycle and even spatial temperature gradients. In a recent work, the authors used an isothermal calorimeter to measure heat flow between developing zebrafish embryos and the surrounding medium [11]. They found that Zebrafish blastula embryos undergo cyclical heating-cooling related to mitotic entry and exit (Fig. 1D). The oscillatory component of the heat flow persists even when inhibitors are used to block DNA replication and mitosis. The authors concluded that the high energetics of the cell cycle are the cost of rapid and coordinated blastomere cell cycles. Surprisingly, spatial gradients of temperature may also exist in the environment of embryos implanted in the placenta [12], a concept that may also be important for embryogenesis of implanted human embryos.

In future studies of embryogenesis, it would also be beneficial to couple molecular tools with embryo temperature controllers. For example, one could consider encoding thermally responsive protein switches in early embryonic systems. The bacterial thermosensor protein TLPA has been used in cell culture to construct heat-sensitive protein-protein interactions [13,14]. The tags constitutively dimerize at room temperature and can be dissociated by heating to 37–42 °C, affording a strategy to release proteins on demand. Combining these synthetic molecular tool strategies – such as tether and release of transcription factors regulating cell differentiation - in addition to the thermal controllers described above offers exciting opportunities for constructing *de novo* patterns in embryos (Fig. 1E).

1.2. I.B. Optogenetic and optochemical regulation of early embryogenesis

Ex vivo fertilization and culture of embryos provide an ideal platform to perturb the natural organization and the timing of inductive events within the embryo using exogenous chemogenic or optical stimulation. In addition, rather than simply observing embryo development, synthetic biology strategies have been developed to induce or repress key regulatory pathways and to record signaling inputs. Using optogenetic dimerization or clustering, researchers have developed strategies in tests of sufficiency to identify critical windows for signaling and spatially vary the action of inducers such as morphogen. These clever tools test the limits of embryonic control systems and have helped to uncover systems properties of embryo patterning.

For many excellent previous reviews on the topic, see published work: [15–17]. In our review we focus specifically on early embryo development, particularly of the blastula.

Protein dimerization via optochemical and optogenetic regulated domains has proven to be a powerful approach to initiate signaling or vary signal amplitude in the embryo. A common strategy is clustering of receptors, such as the BMP receptor, which can activate their downstream signaling. Such an approach was used to photocluster the BMP receptor cytosolic domain and test whether broad spatial induction of the pathway ventralizes the embryo [18] (Fig. 2A). Another common strategy is activation through targeted membrane localization of an upstream pathway component. Wnt signaling can be induced in *Xenopus* embryos via light-mediated dimerization of LRP6-CRY2 conjugated to membrane-localized CIB1 domain [19,20] (Fig. 2B). The researchers demonstrated that this approach was sufficient for the photoinduction of secondary axes in the embryo. A similar tethering strategy was used to optically recruit Raf to the plasma membrane in gastrula stage *Xenopus* whose localized activation induces mesoderm induction and ectopic tail formation [21]. Light-mediated membrane recruitment of a Rho GTPase regulator or phosphoinositide regulators has also been shown sufficient to regulate contractility in *Drosophila* embryos [22,23]. For example, localized reduction of plasma membrane PI(4,5)P2 levels arrests apical constriction and ventral furrow formation, and when coupled to two-photon illumination, enables precise local modulation of contractility.

Separate from optogenetic multimerization, groups have demonstrated modular tools, such as the use of genetic code expansion and photocaged amino acids, to regulate signaling responses in the early embryo with light. Via genetic code expansion, a photocaged lysine was placed in the active site of MEK1 to regulate its activity in the zebrafish early embryo. Upon light stimulation, kinase activity was initiated, promoting embryo elongation and expanding the expression domain of downstream target genes [24]. Additionally, various groups have used the injection of photocaged morpholinos to regulate the timing of gene expression in model embryonic systems [25]. This strategy is complementary to other optogenetic gene circuit approaches, that have been tested in zebrafish [26].

One major advantage of light-inducible systems is their use to identify critical periods of signaling in the embryo, for example, to determine the cell fate necessary for embryo development to larval stages. Using an optoSOS tool to stimulate ERK signaling in *Drosophila* embryos whose endogenous ERK signaling was genetically eliminated, the authors reconstitute *de novo* spatial patterns of pathway activity for defined periods [27] (Fig. 2C). They demonstrated near full recovery of embryonic development by light-mediated stimulation of ERK signaling at the anterior and posterior ends of the embryo and identified threshold levels of signaling in the posterior sufficient to rescue gastrulation. Previously the authors had also demonstrated that light-mediated ERK stimulation was sufficient to switch cells between endoderm and ectoderm cell fates [28]. Additionally, in separate work, they demonstrated the ectopic induction of ERK in an improper region of the embryo was sufficient to suppress ventral furrowing in gastrulation [29]. Finally, as noted above, photocaging of a critical amino acid in the active site of MEK1, revealed a critical period for MEK1-ERK pathway activity during blastula and early gastrula stages of zebrafish embryogenesis [24].

A grand challenge is deconstructing complex gene expression patterns that result from integrated spatial cues of morphogen gradients and signaling responses. By building synthetic architectures to perturb input-output relationships, we will learn fundamental principles of developmental regulation, particularly in the context of complex stimulus-response connections and multicomponent developmental gene networks. In a recent study, the authors stripped away anterior-posterior asymmetries and then transiently optogenetically imposed widespread Bicoid activation to characterize the dynamic response of gap gene transcription [30]. The authors engineered variants of the Bicoid transcription factor whose nuclear localization was photo-controlled. They globally turned on or off Bicoid activity and measured the responsiveness of downstream gene expression. Future experiments will leverage these tools to impose precise spatial synthetic gradients of morphogen activity, including that of Bicoid and others.

Light-regulated control of transcription factors has revealed the inner workings of the regulatory system governing zygotic genome activation through testing of specific hypotheses that could not be accomplished by traditional gene knockdown. Using Cry2 for light-mediated temporal inactivation of the transcription factor Zelda, the authors were able to specifically test whether its activity was required for the period of the minor wave of transcription in NC10–12, the major wave of transcription in NC 13–14, or both [31]. They found that transcriptional output during the major wave depended on Zelda, but that widespread genome activation did not first require a

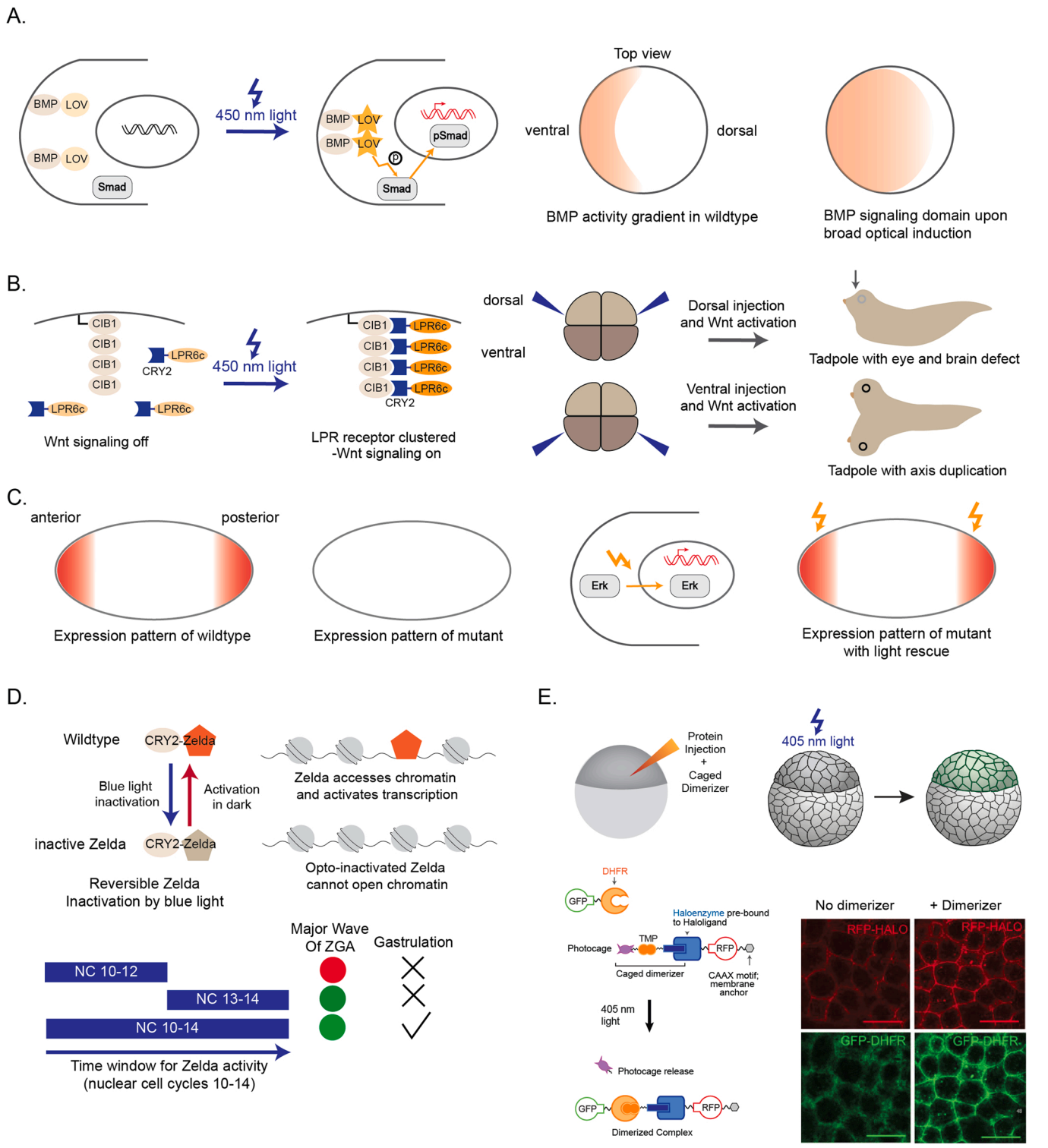

Fig. 2. Optogenetic and optochemical approaches to control developmental signaling and morphogenesis. (A) Light-inducible clustering of the cytosolic side of BMP receptors leads to phosphorylation of Smads and activation of BMP target genes. As a result, the functional spatial domain of BMP signaling is not limited to the ventral side but expands dorsally in zebrafish gastrula embryos. (B) In *Xenopus* embryos, blue light induced clustering of CRY2 conjugated Wnt receptor activates β-catenin signaling at specific times in desired regions. Dorsal Wnt overactivation causes a defect in eye and brain development, while ventral Wnt activation leads to axis duplication. (C) *Drosophila* mutant embryo without Erk activity on anterior or posterior region arrest in gastrulation. Light-inducible Erk activity rescues the developmental arrest. (D) In *Drosophila* blastula embryos, reversible Zelda inactivation identifies separate critical time windows for the proper onset of large-scale genome activation and gastrulation. (E) Conceptual experiment: Injection of purified proteins and a caged dimerizer to induce membrane recruitment upon light activation. Images of *Xenopus* animal pole blastomere at late blastula.

Zelda-dependent minor wave (Fig. 2D). Intriguingly, proper gastrulation required continuous Zelda activity throughout both periods.

Utilizing the large sizes of early embryos, it is possible to manipulate protein levels and localization through microinjection of RNAs and recombinant proteins. However, in very early cleavage stages of vertebrate embryogenesis, for example, over the first few hours post-fertilization, *Xenopus* and zebrafish display slow rates of translation and thus are not compatible with RNA injection to express optogenetic control systems. Instead, future studies will likely utilize the injection of purified proteins containing optochemical dimerization domains along with photocaged dimerizers to modulate protein localization and activity within blastomeres in the early cleavage stages of vertebrates embryogenesis [32,33] (Fig. 2E). Alternatively, it may be possible to sequester and release factors regulating transcription or signaling from preformed membraneless compartments – constructed from coacervating protein injected into the 1-cell embryo, following strategies in cultured cells to control disassembly or client recruitment and releasing using light or heat [14,34,35].

2. Section II. Imposing physical constraints to guide embryo organization

The early embryo is normally subject to several boundary conditions and physical constraints. Cell-cell and cell-ECM interactions produce tension and pulling forces that in turn generate sphericity of the blastula embryo. Many embryos are also surrounded by a protein envelope – the chorion or vitelline membrane – that in addition to its role in sperm entry supports embryo shape prior to hatching. Removal of this physical shell leads large blastula embryos to flatten out due to gravity. Additionally, during gastrulation, signaling cues and gradients drive a massive morphological rearrangement of germ layers. In the past decade, researchers have begun to test the pliability of the embryo – its sensitivity to external forces – such as how its shape influences cell division and self-organization. Further, following a reconstruction approach that synthetic embryology studies have begun to rebuild the complexity of the embryo from a minimal set of cells and patterns. Primarily that has included micropatterning of embryonic stem cells to generate complex 2D and 3D shapes reminiscent of blastula, gastrula, and neurula patterning.

2.1. II.A. Constrained development of early embryos

Early embryos consist of pluripotent blastomeres that initially are quite spherical but also undergo shape changes [3] due to hydraulic expansion of blastocoel [36], and cytoskeletal forces [37] that accompany apical constriction [38]. Pioneering studies aimed to perturb the native shape of the early embryo by encapsulating the blastula in chambers of different shapes [39]. For example, by placing developing sea urchin zygotes into microfabricated wells, the authors found in one-cell stage embryos that nuclei find the long-axis of the embryo, irrespective of shape (Fig. 3A). Through further investigation, the authors found that nuclei position along an axis by microtubules, and this

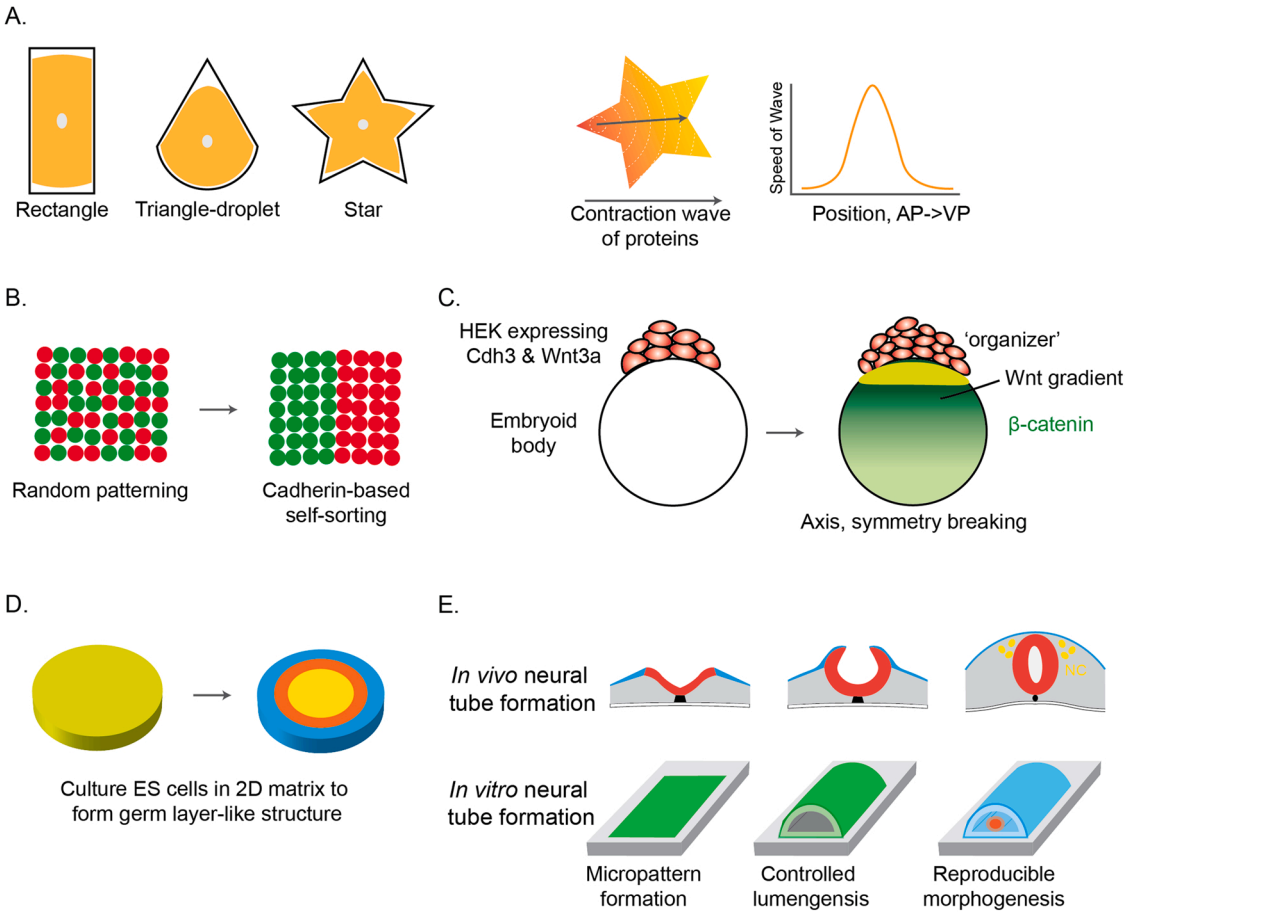

Fig. 3. Micropatterning and physical constraints to guide the formation of embryo-like structure *in vitro*. (A) Microfabricated chambers constrain sea urchin embryos and starfish eggs into defined shapes. The position of nuclei and the dynamics of Rho trigger waves adjust to the distinct cell shape. (B) Mammalian cells expressing distinct sets of cadherin can self-sort into spatially separated subregions. (C) Attachment of HEK cells expressing Cdh3 and Wnt3a to an embryoid can induce a gradient of Wnt β-catenin activity, generating a newly organized axis. (D) Cultured ES cells micropatterned on a 2D matrix with proper inducers can lead to the spontaneous formation of ring-like germ layer patterns. (E) Micropatterning and controllable lumengenesis *in vitro* mimics neural tube formation *in vivo*.

axis also defines the division plane in mitosis. This study illustrated a mechanical approach to define embryo shapes and the robustness of the cell cycle control and division systems to these perturbations.

Similarly, another recent study constrained embryogenesis to analyze how protein dynamics on the membrane adapt to changes in cell shape. Researchers utilized the eggs of starfish *Patiria miniate* to dissect the changes in activity and localization of contraction waves of embryos developed under confinement in compartments [40]. Oocytes are deformed by contraction waves, traveling from vegetal pole to animal pole in the anaphase of meiosis. Although contraction waves are a conserved phenomenon across many species [4], the functional role and the underlying trigger of the waves are less clear. When confined, starfish oocytes quickly adopted different shapes, similar to confined sea urchin zygotes (Fig. 3A). By imaging Rho activity with an rGBD conjugated to GFP, the authors measured wave speed and shape as a function of cell dimension. They concluded that Rho wave initiation is linked to a gradient of cyclinB-Cdk1 that encodes information about cell shape.

2.2. II.B. Assembling minimal embryonic systems via self-organization and templating

Rather than simply observing development events in a complex tissue, synthetic developmental biologists have sought to reconstitute early embryo organization through reconstitution and patterning of a minimal set of cells. Such bottom-up approaches reveal fundamental principles of organization that govern embryogenesis and provide a framework to directly test models for morphogenesis. A key tenet to these strategies is the ability to generate spatially patterned mixtures of cells or to directly micropattern cells and to template their two-dimensional and three-dimensional organization, creating structures often termed embryoids in cell culture. For a comprehensive overview of synthetic embryology and patterning, there are several excellent recent reviews [41,42].

At the simplest level, researchers have sought strategies for cells self-sorting to generate patterned embryoid tissues. Reminiscent of the embryo at gastrulation, cells expressing distinct sets of cadherin can self-sort in culture, whose final patterns of organized structure depend on the starting number of cells and length scale of organization [43] (Fig. 3B). Intriguingly, groups have also sought to impose simplified signaling axes on otherwise isotropic blastuloids via attachment of adherent cells that secrete a signal inducer. For example, the authors induced symmetry breaking in a blastuloid by attachment of HEK cells via P cadherin and then directed Wnt3a secretion to create a gradient of Wnt/β-catenin signaling [44] (Fig. 3C). These embryoid bodies had strong Wnt/β-catenin pathways proximal to the Cdh3-Wnt3a expressing HEK cells, which are reminiscent of an “organizer”.

Due to limitations in the accessibility and availability of human embryos, groups have developed approaches to replicate the early stages of embryogenesis from the differentiation of spatially patterned stem and progenitor cells. An early success was the reconstitution of germ-layer like-assembly from 2D micropatterning of ES cells *in vitro* [45] (Fig. 3D). Moreover, by culturing embryonic stem cells through suspension and shaking, small aggregates of mESCs when properly stimulated can *in vitro* carry out gastrulation-like events including elongation [46]. Further, they can self-organize a pattern of neural, mesodermal, and endodermal cells that mimic gene expression patterns of native embryos. Furthermore, 3D platforms such as matrigel or inverted-pyramidal plates allow cells to aggregate and differentiate into a gastrula-like structure [47,48]. Such gastruloids hold promise as a complementary platform to characterize early mammalian embryo development, paving the way for large-scale genetic analysis or drug test *in vitro*, bypassing sophisticated protocols of culturing precious mouse or human embryos.

With the initial success of generating counterparts of blastocysts and embryos of the gastrula stage, recent studies have focused on recapitulating organogenesis downstream of gastrulation. For example, 3D cultures in matrigel have succeeded in producing the counterpart of epiblast, revealing the role of BMP4 in breaking tissue symmetry [49]. Additionally, neuruloids have been cultured through micropatterning on a chip with the BMP4 signal, extending beyond the simple 2D germ-layer structure to a functional model containing a mixture of neural progenitors, neural crest, and epiblast that holds promise for the study of Huntington’s disease [50]. Neural tube formation is a hallmark of vertebrate development. By developing a chip-based culture system, authors of a cutting-edge study demonstrated that micropatterned stem cells self-organize into precise 3D patterns of cell fate and tissue shapes, displaying controllable lumengenesis and reproducible morphogenesis [51] (Fig. 3E). In complementary work, continuous culturing of gastruloids generates trunk-like organization, which also includes somites and gut, in addition to neural tubes [52]. Notably, patterned Wnt and Nodal signaling has been used to generate a human organizer, capable of inducing an ectopic axis in chick embryos [53], reminiscent of the early transplant experiment of Spermann organizer, which generates a second axis in frog tadpoles [54]. Taken together, strategies for 2D and 3D patterning of embryo-like structures in culture appear poised to offer new biological insights with applications toward improved *in vitro* fertilization (IVF) and drug screening.

Lastly, it is worth highlighting that researchers have also begun to deconstruct the early embryo to characterize its self-organizing properties *ex vivo*. Efforts to characterize blastomeres in a cell-free context primarily focused on the autonomous cell division cycle present in the early blastula and also on subcellular partitioning and scaling of biochemical activities and organelles. For example, autonomous cell division and trigger waves were discovered by extraction of cell-free cytoplasm from oocytes [55,56]. Separately, the encapsulation or compartmentalization of cell-free extracts from *Xenopus* eggs and embryos has revealed how mitotic spindle size and nucleus sizes adapt to blastomere dimensions [57–61]. Although challenging, we envision that in future studies, researchers will leverage these synthetic cell-like blastomeres to reconstruct early events in development, offering a complementary approach to bottom-up cell-patterning strategies.

Declaration of Competing Interest

The authors state they have no competing interests to declare.

Acknowledgements

This work was supported by the National Institute of General Medical Sciences, R35-GM128748 (M.C.G), National Institute of Biomedical Imaging and Bioengineering, R01-EB028320 (M.C.G.) and National Science Foundation MRSEC (DMR1720530) iSuperseed and Seed Awards (M.C.G.). Due to space constraints, we were unable to include citation of many excellent studies and reviews in the field.

References

[1] P.D. Nieuwkoop, J. Faber. Normal Table of Xenopus laevis (Daudin): A Systematical & Chronological Survey of the Development from the Fertilized Egg till the End of Metamorphosis, First ed., Garland Science, 1994.
[2] H. Chen, L.C. Einstein, S.C. Little, M.C. Good, Spatiotemporal patterning of zygotic genome activation in a model vertebrate embryo, Dev. Cell 49 (6) (2019) 852–866. e7.
[3] H. Chen, W. Qian, M.C. Good, Integrating cellular dimensions with cell differentiation during early development, Curr. Opin. Cell Biol. 67 (2020) 109–117.
[4] G.A. Anderson, L. Gelens, J.C. Baker, J.E. Ferrell, Desynchronizing embryonic cell division waves reveals the robustness of xenopus laevis development, Cell Rep. 21 (1) (2017) 37–46.
[5] E.M. Lucchetta, J.H. Lee, L.A. Fu, N.H. Patel, R.F. Ismagilov, Dynamics of Drosophila embryonic patterning network perturbed in space and time using microfluidics, Nature 434 (7037) (2005) 1134–1138.
[6] J. Niemuth, R. Wolf, Developmental asynchrony caused by steep temperature gradients does not impair pattern formation in the wasp, Pimpla turionellae L, Rouxs Arch. Dev. Biol. 204 (7–8) (1995) 444–452.
[7] I.L. Dean, M.E. Shaw, M.A. Tazellar, The Effect of a Temperature Gradient on the Early Development of the Frog, J. Exp. Biol. 5 (4) (1928) 309–336.

[8] S. Kiyonaka, T. Kajimoto, R. Sakaguchi, D. Shinmi, M. Omatsu-Kanbe, H. Matsuura, H. Imamura, T. Yoshizaki, I. Hamachi, T. Morii, Y. Mori, Genetically encoded fluorescent thermosensors visualize subcellular thermoregulation in living cells, Nat. Methods 10 (12) (2013) 1232–1238.
[9] J. Choi, H. Zhou, R. Landig, H.Y. Wu, X. Yu, S.E. Von Stetina, G. Kucsko, S. E. Mango, D.J. Needleman, A.D.T. Samuel, P.C. Maurer, H. Park, M.D. Lukin, Probing and manipulating embryogenesis via nanoscale thermometry and temperature control, Proc. Natl. Acad. Sci. USA 117 (26) (2020) 14636–14641.
[10] M. Fujiwara, S. Sun, A. Dohms, Y. Nishimura, K. Suto, Y. Takezawa, K. Oshimi, L. Zhao, N. Sadzak, Y. Umehara, Y. Teki, N. Komatsu, O. Benson, Y. Shikano, E. Kage-Nakadai, Real-time nanodiamond thermometry probing in vivo thermogenic responses, Sci. Adv. 6 (37) (2020).
[11] J. Rodenfels, K.M. Neugebauer, J. Howard, Heat oscillations driven by the embryonic cell cycle reveal the energetic costs of signaling, Dev. Cell 53 (4) (2020) 492.
[12] S. García-Martínez, R. Latorre, M.A. Sánchez-Hurtado, F.M. Sánchez-Margallo, N. Bernabò, R. Romar, O. López-Albors, P. Coy, Mimicking the temperature gradient between the sow's oviduct and uterus improves in vitro embryo culture output, Mol. Hum. Reprod. 26 (10) (2020) 748–759.
[13] D.I. Piraner, Y. Wu, M.G. Shapiro, Modular thermal control of protein dimerization, ACS Synth. Biol. 8 (10) (2019) 2256–2262.
[14] M.V. Garabedian, W. Wang, J.B. Dabdoub, M. Tong, R.M. Caldwell, W. Benman, B. S. Schuster, A. Deiters, M.C. Good, Designer membraneless organelles sequester native factors for control of cell behavior, Nat. Chem. Biol. 17 (9) (2021) 998–1007.
[15] K.W. Rogers, P. Müller, Optogenetic approaches to investigate spatiotemporal signaling during development, Curr. Top. Dev. Biol. 137 (2020) 37–77.
[16] P.E. Farahani, E.H. Reed, E.J. Underhill, K. Aoki, J.E. Toettcher, Signaling, deconstructed: using optogenetics to dissect and direct information flow in biological systems, Annu Rev. Biomed. Eng. 23 (2021) 61–87.
[17] J. Hartmann, D. Krueger, S. De Renzis, Using optogenetics to tackle systems-level questions of multicellular morphogenesis, Curr. Opin. Cell Biol. 66 (2020) 19–27.
[18] K.W. Rogers, M. ElGamacy, B.M. Jordan, P. Müller, Optogenetic investigation of BMP target gene expression diversity, Elife 9 (2020).
[19] V.V. Krishnamurthy, H. Hwang, J. Fu, J. Yang, K. Zhang, Optogenetic control of the canonical Wnt signaling pathway during xenopus laevis embryonic development, J. Mol. Biol. 433 (18) (2021), 167050.
[20] M.V. Garabedian, M.C. Good, OptoLRP6 illuminates wnt signaling in early embryo development, J. Mol. Biol. 433 (18) (2021), 167053.
[21] V.V. Krishnamurthy, J.S. Khamo, W. Mei, A.J. Turgeon, H.M. Ashraf, P. Mondal, D. B. Patel, N. Risner, E.E. Cho, J. Yang, K. Zhang, Reversible optogenetic control of kinase activity during differentiation and embryonic development, Development 143 (21) (2016) 4085–4094.
[22] G. Guglielmi, J.D. Barry, W. Huber, S. De Renzis, An Optogenetic Method to Modulate Cell Contractility during Tissue Morphogenesis, Dev. Cell 35 (5) (2015) 646–660.
[23] E. Izquierdo, T. Quinkler, S. De Renzis, Guided morphogenesis through optogenetic activation of Rho signalling during early Drosophila embryogenesis, Nat. Commun. 9 (1) (2018) 2366.
[24] J. Liu, J. Hemphill, S. Samanta, M. Tsang, A. Deiters, Genetic code expansion in zebrafish embryos and its application to optical control of cell signaling, J. Am. Chem. Soc. 139 (27) (2017) 9100–9103.
[25] A. Deiters, R.A. Garner, H. Lusic, J.M. Govan, M. Dush, N.M. Nascone-Yoder, J. A. Yoder, Photocaged morpholino oligomers for the light-regulation of gene function in zebrafish and Xenopus embryos, J. Am. Chem. Soc. 132 (44) (2010) 15644–15650.
[26] A. Reade, L.B. Motta-Mena, K.H. Gardner, D.Y. Stainier, O.D. Weiner, S. Woo, TAEL: a zebrafish-optimized optogenetic gene expression system with fine spatial and temporal control, Development 144 (2) (2017) 345–355.
[27] H.E. Johnson, N.J.V. Djabrayan, S.Y. Shvartsman, J.E. Toettcher, Optogenetic Rescue of a Patterning Mutant, Curr. Biol. 30 (17) (2020) 3414–3424.e3.
[28] H.E. Johnson, J.E. Toettcher, Signaling dynamics control cell fate in the early drosophila embryo, Dev. Cell 48 (3) (2019) 361–370.e3.
[29] S. McFann, S. Dutta, J.E. Toettcher, S.Y. Shvartsman, Temporal integration of inductive cues on the way to gastrulation, Proc. Natl. Acad. Sci. USA 118 (23) (2021).
[30] A. Singh, W. Ping, S. Ryabichko, Jo Raimundo, M. Swan, E. Wieschaus, T. Gregor, J. Toettcher, Optogenetic control of the Bicoid morphogen reveals fast and slow modes of gap gene regulation, bioRxiv (2021), https://doi.org/10.1101/2021.10.13.464280.
[31] S.L. McDaniel, T.J. Gibson, K.N. Schulz, M. Fernandez Garcia, M. Nevil, S.U. Jain, P.W. Lewis, K.S. Zaret, M.M. Harrison, Continued activity of the pioneer factor zelda is required to drive zygotic genome activation, Mol. Cell 74 (1) (2019) 185–195.e4.
[32] J.G. Bermudez, A. Deiters, M.C. Good, Patterning microtubule network organization reshapes cell-like compartments, ACS Synth. Biol. 10 (6) (2021) 1338–1350.
[33] R.M. Caldwell, J.G. Bermudez, D. Thai, C. Aonbangkhen, B.S. Schuster, T. Courtney, A. Deiters, D.A. Hammer, D.M. Chenoweth, M.C. Good, Optochemical control of protein localization and activity within cell-like compartments, Biochemistry 57 (18) (2018) 2590–2596.
[34] E.H. Reed, B.S. Schuster, M.C. Good, D.A. Hammer, SPLIT: stable protein coacervation using a light induced transition, ACS Synth. Biol. 9 (3) (2020) 500–507.
[35] B.S. Schuster, E.H. Reed, R. Parthasarathy, C.N. Jahnke, R.M. Caldwell, J. G. Bermudez, H. Ramage, M.C. Good, D.A. Hammer, Controllable protein phase separation and modular recruitment to form responsive membraneless organelles, Nat. Commun. 9 (1) (2018) 2985.
[36] C.J. Chan, M. Costanzo, T. Ruiz-Herrero, G. Mönke, R.J. Petrie, M. Bergert, A. Diz-Muñoz, L. Mahadevan, T. Hiiragi, Hydraulic control of mammalian embryo size and cell fate, Nature 571 (7763) (2019) 112–116.
[37] H.Y.G. Lim, N. Plachta, Cytoskeletal control of early mammalian development, Nat. Rev. Mol. Cell Biol. 22 (8) (2021) 548–562.
[38] C.R. Samarage, M.D. White, Y.D. Álvarez, J.C. Fierro-González, Y. Henon, E. C. Jesudason, S. Bissiere, A. Fouras, N. Plachta, Cortical tension allocates the first inner cells of the mammalian embryo, Dev. Cell 34 (4) (2015) 435–447.
[39] N. Minc, D. Burgess, F. Chang, Influence of cell geometry on division-plane positioning, Cell 144 (3) (2011) 414–426.
[40] M. Wigbers, T.H. Tan, F. Brauns, J. Liu, S.Z. Swartz, E. Frey, N. Fakhri, A hierarchy of protein patterns robustly decodes cell shape information, Nat. Phys. (2021) 578–584.
[41] J.J. Metzger, M. Simunovic, A.H. Brivanlou, Synthetic embryology: controlling geometry to model early mammalian development, Curr. Opin. Genet Dev. 52 (2018) 86–91.
[42] G. Blin, Quantitative developmental biology in vitro using micropatterning, Development 148 (15) (2021).
[43] E. Cachat, W. Liu, K.C. Martin, X. Yuan, H. Yin, P. Hohenstein, J.A. Davies, 2- and 3-dimensional synthetic large-scale de novo patterning by mammalian cells through phase separation, Sci. Rep. 6 (2016) 20664.
[44] F. Glykofrydis, E. Cachat, I. Berzanskyte, E. Dzierzak, J.A. Davies, Bioengineering self-organizing signaling centers to control embryoid body pattern elaboration, ACS Synth. Biol. 10 (6) (2021) 1465–1480.
[45] A. Warmflash, B. Sorre, F. Etoc, E.D. Siggia, A.H. Brivanlou, A method to recapitulate early embryonic spatial patterning in human embryonic stem cells, Nat. Methods 11 (8) (2014) 847–854.
[46] L. Beccari, N. Moris, M. Girgin, D.A. Turner, P. Baillie-Johnson, A.C. Cossy, M. P. Lutolf, D. Duboule, A.M. Arias, Multi-axial self-organization properties of mouse embryonic stem cells into gastruloids, Nature 562 (7726) (2018) 272–276.
[47] B. Sozen, G. Amadei, A. Cox, R. Wang, E. Na, S. Czukiewska, L. Chappell, T. Voet, G. Michel, N. Jing, D.M. Glover, M. Zernicka-Goetz, Self-assembly of embryonic and two extra-embryonic stem cell types into gastrulating embryo-like structures, Nat. Cell Biol. 20 (8) (2018) 979–989.
[48] S. Chhabra, L. Liu, R. Goh, X. Kong, A. Warmflash, Dissecting the dynamics of signaling events in the BMP, WNT, and NODAL cascade during self-organized fate patterning in human gastruloids, PLoS Biol. 17 (10) (2019), e3000498.
[49] M. Simunovic, J.J. Metzger, F. Etoc, A. Yoney, A. Ruzo, I. Martyn, G. Croft, D. S. You, A.H. Brivanlou, E.D. Siggia, A 3D model of a human epiblast reveals BMP4-driven symmetry breaking, Nat. Cell Biol. 21 (7) (2019) 900–910.
[50] T. Haremaki, J.J. Metzger, T. Rito, M.Z. Ozair, F. Etoc, A.H. Brivanlou, Self-organizing neuruloids model developmental aspects of Huntington's disease in the ectodermal compartment, Nat. Biotechnol. 37 (10) (2019) 1198–1208.
[51] E. Karzbrun, A.H. Khankhel, H.C. Megale, S.M.K. Glasauer, Y. Wyle, G. Britton, A. Warmflash, K.S. Kosik, E.D. Siggia, B.I. Shraiman, S.J. Streichan, Human neural tube morphogenesis in vitro by geometric constraints, Nature 599 (7884) (2021) 268–272.
[52] J.V. Veenvliet, A. Bolondi, H. Kretzmer, L. Haut, M. Scholze-Wittler, D. Schifferl, F. Koch, L. Guignard, A.S. Kumar, M. Pustet, S. Heimann, R. Buschow, L. Wittler, B. Timmermann, A. Meissner, B.G. Herrmann, Mouse embryonic stem cells self-organize into trunk-like structures with neural tube and somites, Science 370 (6522) (2020).
[53] I. Martyn, T.Y. Kanno, A. Ruzo, E.D. Siggia, A.H. Brivanlou, Self-organization of a human organizer by combined Wnt and Nodal signalling, Nature 558 (7708) (2018) 132–135.
[54] R. Harland, J. Gerhart, Formation and function of Spemann's organizer, Annu Rev. Cell Dev. Biol. 13 (1997) 611–667.
[55] J.B. Chang, J.E. Ferrell, Mitotic trigger waves and the spatial coordination of the Xenopus cell cycle, Nature 500 (7464) (2013) 603–607.
[56] X. Cheng, J.E. Ferrell, Spontaneous emergence of cell-like organization in, Science 366 (6465) (2019) 631–637.
[57] S. Gai, C. Li, P. Yang, J. Lin, Recent progress in rare earth micro/nanocrystals: soft chemical synthesis, luminescent properties, and biomedical applications, Chem. Rev. 114 (4) (2014) 2343–2389.
[58] M.C. Good, Encapsulation of xenopus egg and embryo extract spindle assembly reactions in synthetic cell-like compartments with tunable size, Methods Mol. Biol. 1413 (2016) 87–108.
[59] C. Brownlee, R. Heald, Importin α partitioning to the plasma membrane regulates intracellular scaling, Cell 176 (4) (2019) 805–815.e8.
[60] Y. Hara, C.A. Merten, Dynein-based accumulation of membranes regulates nuclear expansion in xenopus laevis egg extracts, Dev. Cell 33 (5) (2015) 562–575.
[61] R.N. Mukherjee, J. Sallé, S. Dmitrieff, K.M. Nelson, J. Oakey, N. Minc, D.L. Levy, The perinuclear ER scales nuclear size independently of cell size in early embryos, Dev. Cell 54 (3) (2020) 395–409.e7.