Advanced Review

Models of convergent extension during morphogenesis

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Convergent extension (CE) is a fundamental and conserved collective cell movement that forms elongated tissues during embryonic development. Thus far, studies have demonstrated two different mechanistic models of collective cell movements during CE. The first, termed the crawling mode, was discovered in the process of notochord formation in *Xenopus laevis* embryos, and has been the established model of CE for decades. The second model, known as the contraction mode, was originally reported in studies of germband extension in *Drosophila melanogaster* embryos and was recently demonstrated to be a conserved mechanism of CE among tissues and stages of development across species. This review summarizes the two modes of CE by focusing on the differences in cytoskeletal behaviors and relative expression of cell adhesion molecules. The upstream molecules regulating these machineries are also discussed. There are abundant studies of notochord formation in *X. laevis* embryos, as this was one of the pioneering model systems in this field. Therefore, the present review discusses these findings as an approach to the fundamental biological question of collective cell regulation.

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INTRODUCTION

Convergent Extension (CE): A Conserved Cellular Movement During Morphogenesis

Convergent extension (CE) is a cellular process conserved across different species, as well as in different tissues and stages of development. During the CE process, cells sense the global, tissue-level planar polarity. They will subsequently intercalate with each other to converge as the long axis of the tissue forms. As a consequence, the width of the developing tissue narrows as the length increases (Figure 1(a)). This was originally observed in a study of notochord formation in the *Xenopus laevis* embryo,[1,2] and has been investigated extensively in subsequent studies of CE during notochord formations in *X. laevis*, zebrafish (*Danio rerio*), and *Ciona intestinalis* embryos.[1,3–6] In addition to notochord formation, CE is also observed during other morphogenetic events that occur at later stages of development, such as the elongation of the neural plate in *X. laevis*,[7] chick,[8] and mouse embryos[9,10]; the formation of the kidney tubule in *X. laevis* embryos[11]; and the cochlea in mouse embryos.[12] Currently ongoing studies investigate the role of CE in other tissue development, spearheaded by a recent study demonstrating its role in the formation of the mouth in *X. laevis* embryos.[13] Considering the conservation of CE across multiple species, diverse tissue types and throughout various stages of morphogenesis, understanding the cellular and molecular mechanisms underlying CE is of paramount importance in the field of morphogenesis.

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Pioneering Model of CE: Notochord Formation in *X. laevis* Embryo

Although the entire mesoderm converges and extends during gastrulation, the most extreme convergence occurs in the presumptive notochord, which made it

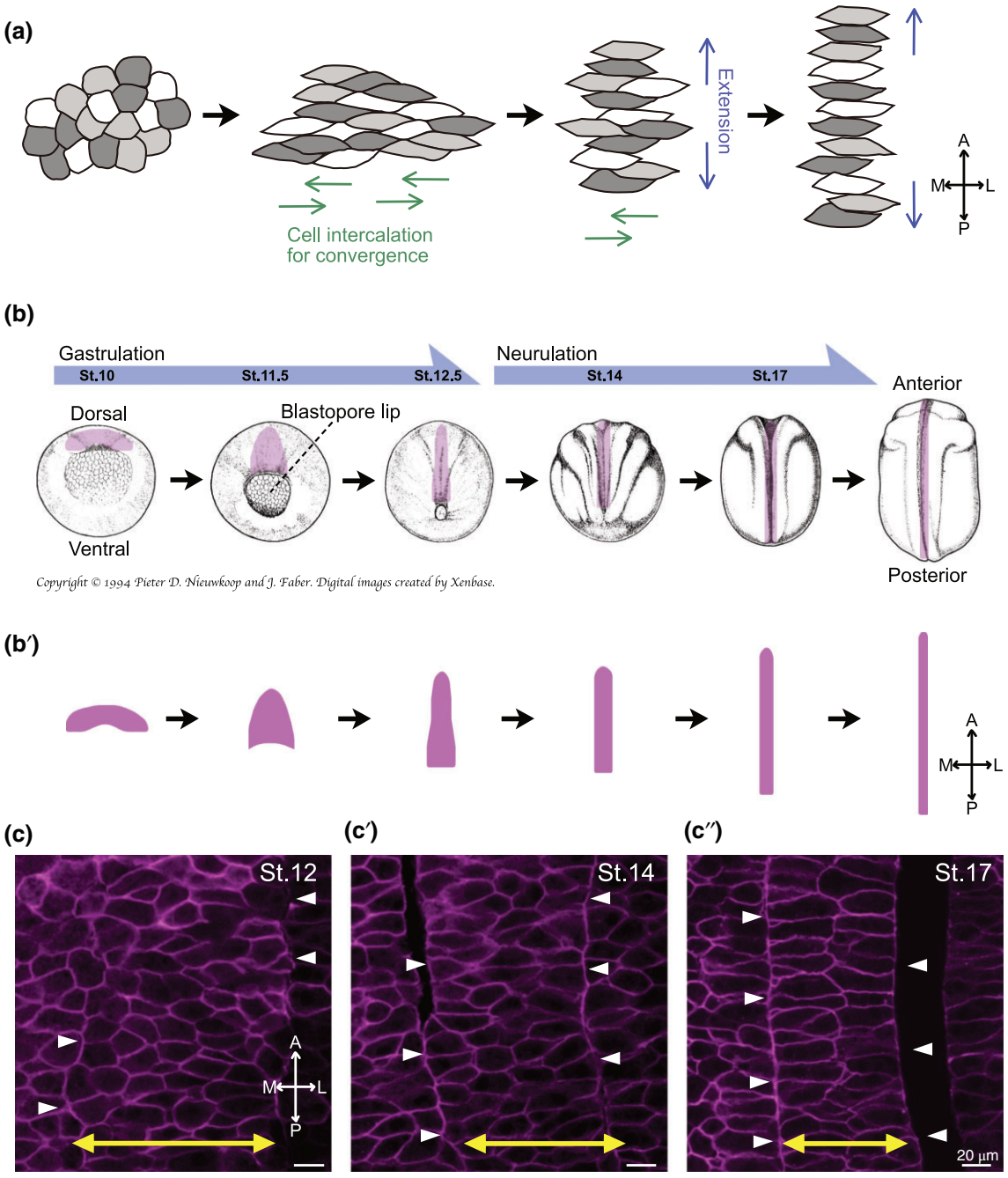

FIGURE 1 | Convergent extension (CE) during the formation of *Xenopus laevis* notochord. (a) General cell movements exhibited during CE. The cells move bidirectionally along the future short axis of the elongating tissue (horizontal axis in this scheme, green arrows) and intercalate between each other. The continuous intercalation allows the tissue to elongate along the perpendicular axis (blue arrows). (b, b′) Notochord formation during gastrulation in the *X. laevis* embryo. The region that develops into the notochord is marked with a pink color. The notochord elongates along the anteroposterior axis of the embryo by cells intercalating along the mediolateral axis. (c–c") Immunostaining of embryos injected with membrane-GFP mRNA. The notochord dramatically narrows during neurulation. Arrowheads indicate notochord–somite boundary, and the yellow arrows indicate the width of the notochord. A, anterior; P, posterior; M, medial; L, lateral; St, embryonic stage.

the pioneering model for CE. Notochord formation in *X. laevis* embryos is the longest-standing model of CE, because of its favorability for microscopic observations of CE in explants (Figure 1). Notochord cells during CE elongate along the mediolateral axis, and the tissue shape becomes narrower and longer as the cells intercalate with each other through gastrulation to neurulation (Figure 1(b), (b′), (c)–(c″)). Tissue

explants isolated from a particular region of *X. laevis* embryos maintain normal development as they would in an intact embryo. This feature enables researchers to observe cell behaviors in tissues such as the notochord, located in the deeper layers of the embryo.

Studies using isolated tissue explants from the notochord region, referred to as Keller explants, have contributed to the accumulation of information on basic cellular behavior during CE[1,14] (Figure 8(a)). Keller explants permit the large-scale analysis of gene expression or protein expression during CE.[15] Moreover, *X. laevis* embryos have relatively large cell size (30–50 μm diameter in the *X*–*Y* plane), which allows the visualization of cellular and intracellular behaviors during CE. These large-sized cells of Keller explants, together with the establishment of live imaging technologies, have permitted observation of cellular and intracellular behaviors in real time. On the basis of these useful technical systems, researchers have used *X. laevis* embryos to investigate the cellular and molecular mechanisms of the CE process.

Two Modes for the Cellular Intercalation During CE: Crawling and Contraction

The first model of CE, the crawling mode, was proposed in the mid-1980s from studies of the *X. laevis* notochord (Figure 2(a)).[16] Cells in the *X. laevis* notochord utilize membrane protrusions at both tips of the elongated cells to crawl through spaces between neighboring cells and cause cellular intercalation.[17,18] During the crawling mode, cells are able

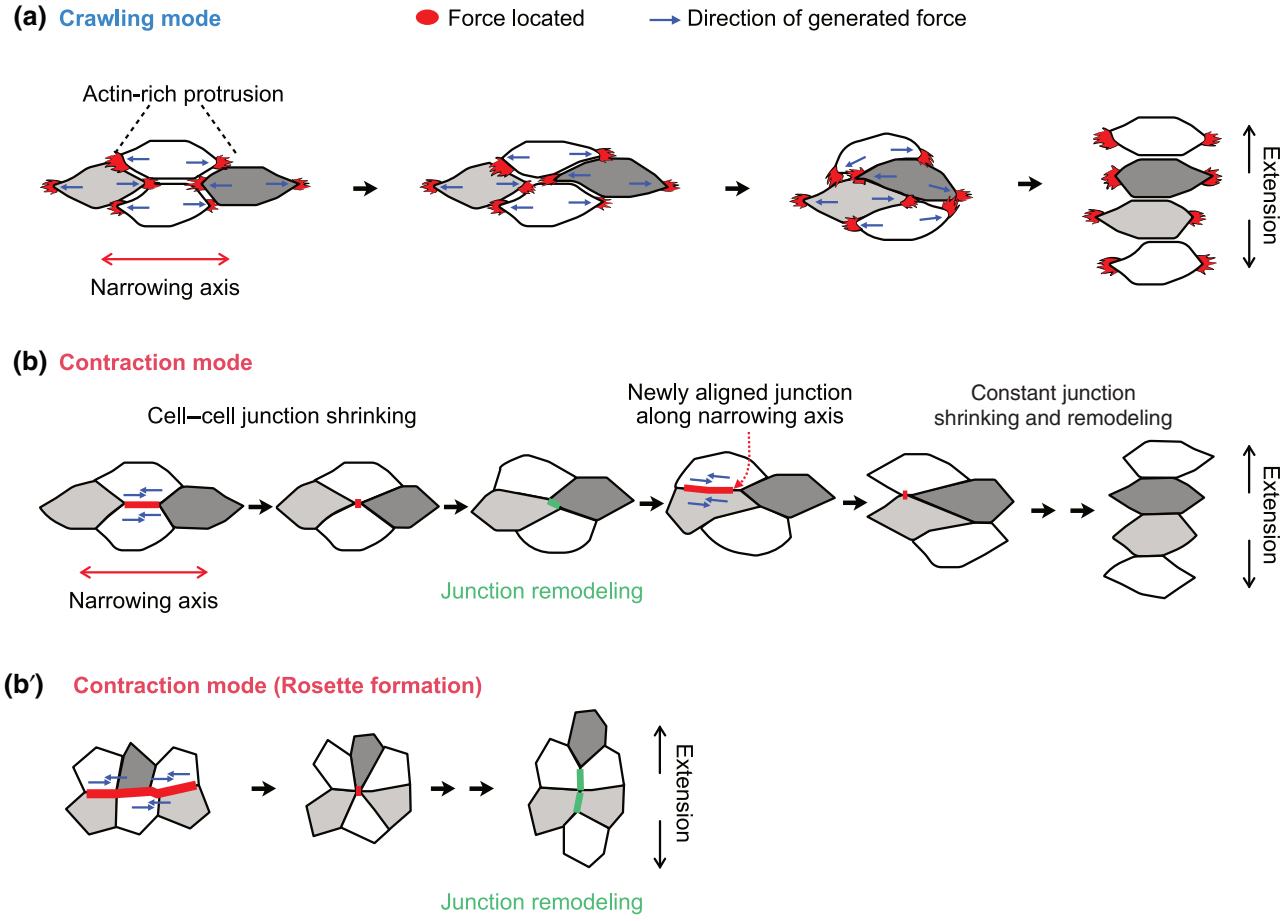

FIGURE 2 | Two modes of convergent extension. (a) Driving force and cell movements in the crawling mode. The cells elongate along the mediolateral axis (short axis of the elongating tissue) and crawl and tug the neighboring cells by actin-rich protrusions located at both tips of the elongated cells (red parts) that lead to the intercalating movements. (b, b′) Driving force and cell movements in the contraction mode. Activation of actomyosin along the cell–cell junction constricts the junction (red line) and pulls the neighboring cells (dark and light gray colored cells) to the center in this scheme. The actomyosin is activated at the cell–cell junction aligning along the short axis of the elongating cell. After the neighboring cells meet together, these two cells construct a new cell–cell junction (junction remodeling, green short line), and actomyosin starts constricting the other cell–cell junction aligned to the short axis of the tissue. Repeating these events allows the cell to establish intercalating movements. (B′) is a scheme of rosette formation.

to move bidirectionally along the mediolateral axis (narrowing axis in Figure 2) following these 'bipolar' membrane protrusions[19] (Figure 2(a), blue arrows).

The bipolar membrane protrusions were initially observed through compound microscopy or scanning electron microscopy in fixed *X. laevis* embryos.[2] Confocal microscopy captured a clearer view of the membrane protrusions[20] and the enrichment of actin filaments in these membrane protrusions,[21,22] which resemble lamellipodia in migrating cultured cells. These imaging studies also revealed the actin filament network spreading through the cells in the same plane with membrane protrusions (Figure 3(a), orange lines). The actin filament network exhibits synchronized oscillation with the contraction of the cell surface, suggesting that the combination of contraction and crawling protrusions exerts traction forces between the cells to cause mediolateral intercalation (Figure 3(a)). On the basis of an array of studies, this crawling mode has become one of the hallmark cell movements associated with CE in mesenchymal tissues such as the notochord.

In the early 2000s, another model of CE, the contraction mode was discovered in *Drosophila melanogaster* embryos during germband extension, a process of epithelial tissue elongation.[23–25] In this mode, cell–cell junctions align in a parallel fashion to the long axis of the tissue (i.e., vertically aligned

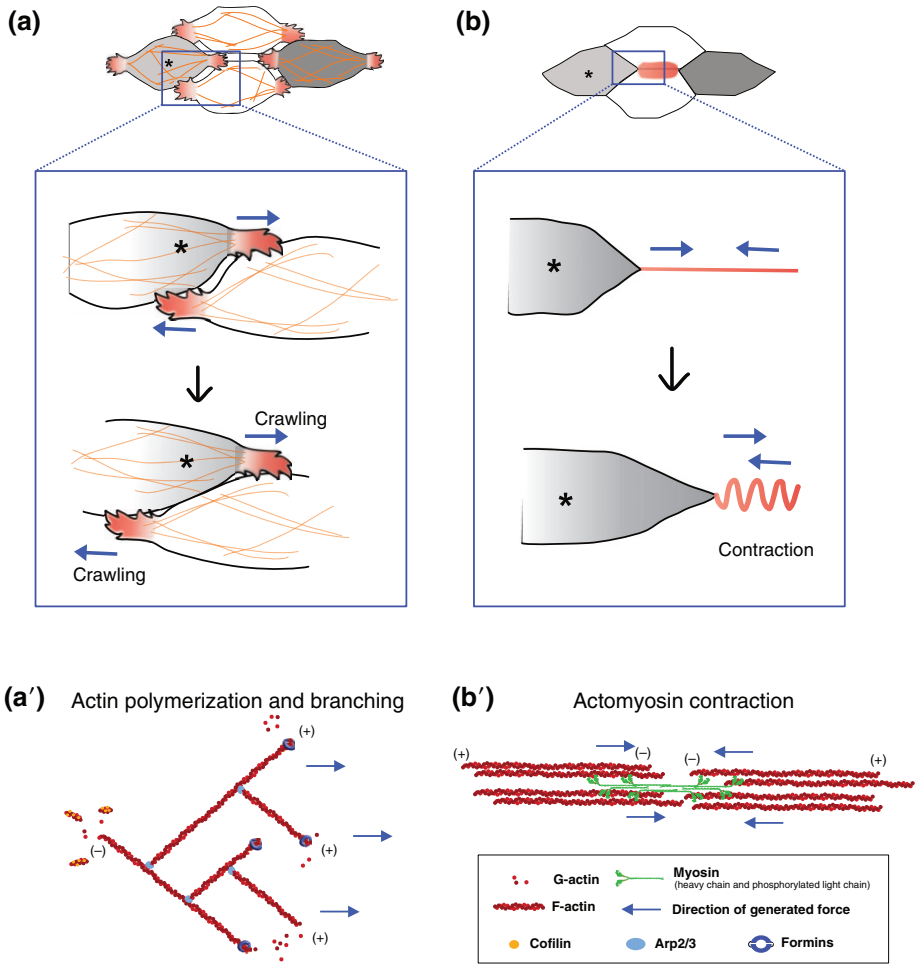

FIGURE 3 | Driving forces of the two modes of convergent extension. (a) Actin-rich protrusions allow the cell to crawl along the neighboring cells. Actin is polymerized at the protrusions and pushes the membrane forward between the neighboring cells. The actin cables provide the resistant force for cell elongation, in order for the cells to tug the adjacent cells by the crawling motion. In this model, the cells move actively. (a′) A model of actin filament polymerization and branching. Actin-binding proteins such as Arp2/3, Formin, or Cofilin function to branch, polymerize or sever the actin filament. (b) Actomyosin activation constricts the cell–cell junction. The neighboring cell (gray colored) is pulled by the shrinking cell–cell junction. In this model, the cells move passively. (b′) A model of actomyosin contraction. Phosphorylation of myosin light chain triggers the actin filaments to slide toward the myosin complex.

junction) where they accumulate actomyosin (the functional complex of actin and myosin, Figure 3 (b′)). The activation of actomyosin along the cell–cell junctions contracts the cell membrane, and the resulting shrinkage produces the necessary force to move the neighboring cells. Interestingly, another actomyosin population called medial actomyosin is known to constrict the apical surface of cells, and its behavior is also associated with the contraction of cell–cell junctions.[26,27] This contraction mode was also confirmed in epithelial tissues of other model systems discussed later in this review.

These two modes were originally considered as distinct cellular behaviors in mesenchymal or epithelial tissues. However, recently, the contraction mode was observed in CE of the *X. laevis* notochord,[28] which had previously been the definitive model for the crawling mode. Similarly to CE in the germband, cell–cell junctions aligning along the mediolateral axis of the notochord contain actomyosin, and their contraction generates forces that pull on the neighboring cells (Figure 2(b)). These neighboring cells subsequently generate a new cell–cell junction and undergo junction remodeling (Figure 2(b), green line). Following the completion of these topological changes, another cell–cell junction aligned vertically initiates a new shrinking process (Figure 2(b), second red line). Surprisingly, very recently, the crawling mode has also been observed in germband extension—a model of the contraction mode of CE.[29] These new discoveries propose that cells may adopt multiple modes to manage their complex collective cell movement, as first suggested by the study of mouse neural plate.[9]

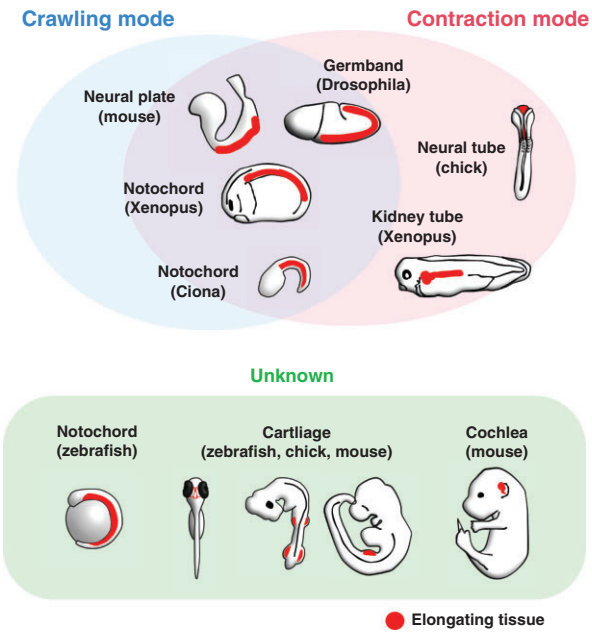

FIGURE 4 | Various tissues showing convergent extension. Classification of CE models based on the crawling and contraction modes. Membrane protrusions are observed during CE in the mouse neural plate, *Xenopus laevis* notochord, and *Ciona intestinalis* notochord (i.e., crawling mode: blue circle). The formation of *X. laevis* notochord also exhibits the contraction mode, and it is thought that the mouse neural plate and *C. intestinalis* notochord also display the contraction mode. Cell–cell junction contractions are observed during CE in *Drosophila melanogaster* germband extension, chick neural tube elongation, and *X. laevis* kidney tubule elongation (i.e., contraction mode: red circle). Although there are other developmental processes showing CE, their modes are unclear (green box).

MAIN TOPICS AND QUESTIONS DISCUSSED IN THIS REVIEW

The biological significance of the employment of two distinct modes of CE remains unclear. It has been hypothesized that mesenchymal tissues, most of which eventually differentiate into adult connective tissue, adapted the crawling mode because of the inherent weak adhesive forces existing in mesenchymal cells. On the other hand, epithelial tissues are thought to utilize the contraction mode because of the strong adhesive forces existing in tight junctions and adherens junctions in epithelial cells. The coexistence of these two modes in a single tissue suggests greater flexibility in the mechanism, and requires consideration of additional interpretations.

Another fundamental question regarding collective cell movement in the CE process is the simultaneous movement of multiple cells without disturbing the anatomical integrity of the tissue. To achieve coordinated collective cell movement, accurate crosstalk between the cytoskeletons and adhesion components under the regulation of molecular signaling between cells is indispensable. However, the actual interactions between the cytoskeletons and cell adhesion components remain unclear in both the crawling and contraction modes. Furthermore, regardless of the number of pathways and associated sets of molecules suggested to be involved in CE, the interplay between these molecules remains only partially understood.

This review discusses both modes of CE in various model organisms, and focuses on cell movements elicited by cytoskeleton dynamics, participation of cell adhesion molecules, and involvement of upstream regulatory molecules. In addition, the specific microenvironment that impacts these two modes during the morphogenesis of particular tissues, as well as the collective cell coordination that occurs

across tissues in the case study of *X. laevis* notochord formation is discussed.

Cytoskeletons Involved in Crawling and Contraction Modes of CE

The actin cytoskeleton provides a major driving force for general cell migration, and its role as the major contributor to cell movement during CE is no exception. During the crawling and contraction modes illustrated in Figure 2, the cytoskeleton appears to behave differently during the progression of cell intercalation to achieve overall tissue convergence.

Crawling by Bipolar Actin-Rich Membrane Protrusions

In the crawling mode, the cells grip each other and crawl along the neighboring cells using the actin-rich membrane protrusions located at both tips of the elongated cells (Figure 3(a)).[2,17] Despite the significant role of the bipolar membrane protrusions, the underlying cytoskeletal regulation of protrusive activity and traction has not been yet fully elucidated.

Actin turnover in membrane protrusions may be a pivotal cytoskeletal behavior for the crawling mode. In general, crawling movements that exploit membrane structures such as lamellipodia are thought to be driven by the turnover of the actin cytoskeleton, through continuous polymerization and depolymerization.[30–32] Rho and Rac, two members of the Ras superfamily of guanosine triphosphatases (GTPases),[33,34] regulate actin polymerization and branching by controlling actin-binding proteins such as Arp2/3,[35] disheveled-associated activator of morphogenesis 1 (Daam1, a Formin family),[36,37] Diaphanous,[38] and Cofilin[39] (Figure 3(a′)). Previous studies on the *X. laevis* notochord have shown that Rho and Rac are required for CE, as shown by the disturbance of membrane protrusions and reduced CE in the embryos overexpressing dominant negative forms.[40] It is noteworthy that the membrane protrusions recently discovered through the study of *D. melanogaster* germband CE, require Rac activity to extend the protrusion, suggesting that the actin polymerization contributes to the protrusive activity.[29] On the basis of these studies of CE, these general regulators of actin turnover may regulate bipolar membrane protrusions during CE.

Membrane protrusions may not be able to cause CE independently of other factors. Continuous pushing of the membrane protrusions by actin turnover at both tips of the cell, without sufficient cell stiffness (lack of elasticity) would lead to continuous cell elongation, without net directional intercalation. If there are differences in protrusive activities between the two ends of the moving cell, then additional factors may be required to determine the direction of movement. The studies on the formation of the notochord in *X. laevis* explained that actomyosin contraction throughout the cell cortex, referred to as punctuated actin contraction,[22] provides the forces to resist cell elongation. Subsequently, the cortical actomyosin contraction generates traction forces enabling cells to haul on each other[41] (Figure 2(a)). In *D. melanogaster*, membrane protrusions were observed only at the basal side of the cell, whereas the apical side manifests junctional contraction.[29] It is therefore suggested that membrane protrusions accomplish cellular intercalation in combination with other cytoskeletal behaviors such as actomyosin contraction.

In addition to CE in the *D. melanogaster* germband, the polarized protrusive activity at the membrane (evidence of the crawling mode in CE) was found in several other model systems such as CE in *C. intestinalis* notochord[4,42] and in mouse neural plate[9] formation (Figure 4, in blue circle). These findings demonstrate that the crawling mode is a conserved morphogenetic process of both invertebrates and vertebrates.

Contraction of Cell–Cell Junctions by Actomyosin Activation

In the contraction mode, the myosin light chain is predominantly phosphorylated at the cell–cell junctions along the narrowing/short axis of the elongating tissue (Figures 2(b) and 3(b)). The short axis is along the dorsoventral axis in the case of *D. melanogaster* germband, and along the mediolateral axis in the *X. laevis* notochord. Subsequently, the contractile forces generated by the activation of actomyosin (Figure 3(b′)) shrink the junctions to pull the neighboring cells inward, resulting in cellular intercalation along with junctional remodeling. Germband extension studies in *D. melanogaster* showed that contraction occurs simultaneously at single cell–cell and multiple cell junctions in a rosette formation (Figure 2(b′)).[43,44] The constant junction shortening and remodeling enable the developing tissue to elongate as cells are pulled passively by the shortening cell–cell junction of the neighboring cells, instead of being controlled autonomously, as in the crawling model.

Coupled with the contraction of junctional actomyosin, another population of actomyosin, termed medial actomyosin, was reported through the observation of apical surfaces of cells in the germband.[26,27] Medial actomyosin oscillates and flows

toward the shrinking cell junctions, and this oscillation is synchronized with cell junction shortening. Similar to the crawling mode in the formation of the *X. laevis* notochord, where both lamellipodial and cortical pools of actin are employed, the combination of multiple cytoskeletal pools of actomyosin may also be required for the contraction mode.

The contraction mode is a well-conserved CE process for epithelial tissues observed not only in *D. melanogaster* but also in several other species and contexts, including kidney tubule elongation in *X. laevis* embryos[11]; neural tube development in chick embryos[8]; and primitive streak formation in chick embryos[45] (Figure 4, in red circle). The contraction mode, usually evident by the localization of actomyosin, plays a role in CE processes also known to exhibit the crawling mode such as in *X. laevis* notochord formation,[28] in mouse neural plate,[9] and in *C. intestinalis* notochord development[46] (Figure 4, overlap between blue and red circle). Many other tissues exhibit elongation of tissues during development. However, currently, the role of these CE modes on the morphogenesis of tissues remains unknown (Figure 4, green square).

Cell Adhesion Characteristics of Crawling and Contraction Modes of CE

Both modes of CE require a distinct set of adhesive properties to elicit a unique cell movement. Although the significance of cell adhesion in the coordination of mechanical forces required to bring together multiple types of cell movement is acknowledged, information regarding the identity of the adhesion molecules during CE is still disputed. Furthermore, the role of cell–matrix adhesion, which is a critical property of the migrating single cell *in vitro*, is not well understood in the CE process. In this section, current insights regarding adhesion proteins and their involvement in each CE model are discussed.

Weakening of the Adhesive Property During the Crawling Mode

In the crawling mode, cells need to squeeze between neighboring cells that supposedly loosen as their adhesive forces weaken during cell movement (Figure 5(a)). C-cadherin (Cadherin-3, P-cadherin) at the cell–cell junctions is internalized by endocytosis at the initiation of gastrulation in *X. laevis* embryos.[47] A recent study showed that the remaining C-cadherin after internalization is spatially localized exclusively at small sites on the membrane protrusions during CE in *X. laevis* notochord formation.[48] These findings suggest that cadherin function may still be present at these membrane protrusions, which would allow the crawling cells to remain adhered weakly and dynamically to their neighboring cells (so-called 'kiss points'[48]).

Another candidate protein for involvement in cell adhesion in the crawling mode is protocadherin, a member of the cadherin superfamily and originally found to be expressed in the nervous system.[49] Several reports have suggested that the role of paraxial protocadherin (Papc, protocadherin 8) as an adhesion molecule is especially important during the early phase of CE in the process of *X. laevis* mesoderm including notochord. It is worth noting that protocadherin has considerably weaker adhesive properties,[50] making this molecule a more attractive candidate because it is amenable to the adhesive flexibility presumed to be required in the crawling mode.

Several reports about Papc suggest that its cell adhesion properties may be transformed during the process of CE. Papc is expressed specifically in the paraxial mesoderm (somite) during the neurula stage of *X. laevis*[51] and zebrafish[52] embryonic development. However, it is detected also in the axial mesoderm (notochord) in the early gastrula.[51] Importantly, it has recently been shown that Papc is localized at the cell membrane in the early phase of CE in *X. laevis* notochord formation. However, its localization at the membrane is subsequently disrupted via ubiquitination.[53] This observation suggests that whereas Papc may function in the earlier phase of CE, another

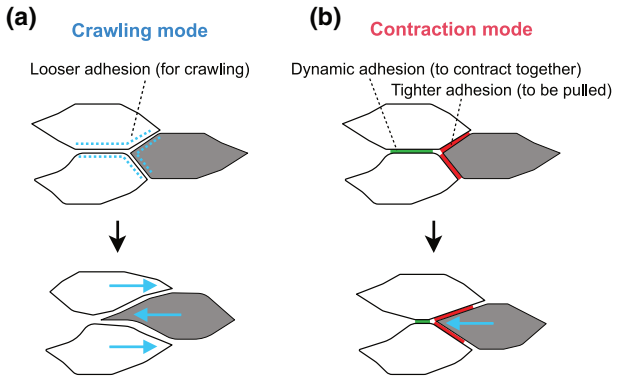

FIGURE 5 | Cell adhesion properties for the two modes of convergent extension. (a) Cell adhesion properties suggested for the crawling mode. Loose adhesion is required for the cell (colored gray) to crawl between neighboring cells (colored white). (b) Cell adhesion properties suggested for the contraction mode. Dynamic adhesion (green line) is required for the cells (colored white) to attach together and shrink the shared cell–cell junction, while not impeding contraction. Tighter adhesion (red line) between the cells exhibiting cell–cell junction shrinkage (white) and the cells consequently being pulled (colored gray) is required for the cell (gray) to be pulled by the contraction occurring between cells (white).

adhesion molecule may be responsible for the cell–cell adhesion maintained throughout CE.

Interestingly, the functional and mechanical properties of the extracellular matrix (ECM) are also altered during CE in *X. laevis* notochord formation. Integrin–fibronectin interaction is significant for cellular intercalation movements during CE.[54] Fibronectin is found on the blastocoel roof, where notochord cells migrate toward the anterior side during CE[55]; therefore, it may be a substrate which elicits CE movement in notochord cells. Fibrillin is another essential component of ECM, which gradually accumulates around the notochord during CE in *X. laevis* notochord formation.[56,57]

The transition taking place to modify cell-adhesive properties, such as Papc degradation and secretion of fibrillin, may play a significant role in preferentially establishing each CE mode. Because Papc expresses only in the earlier phase of CE, the crawling mode, which presumably requires weak adhesion properties, may work in same phase. Indeed, the bipolar membrane protrusions, which facilitate cell crawling, are usually more prominent during the early phase, whereas cell junction shrinkage is more visible during the late phase of CE in notochord formation.

Dynamic Regulation of Cell Adhesion in the Contraction Mode

In contrast to protocadherins, cadherins play significant roles in cell–cell adhesion during CE mediated by the contraction mode. A study of germband extension in *D. melanogaster* showed that E-cadherin is required for medial actomyosin flow,[26] and exhibits an oscillation pattern synchronized to actomyosin pulses at the shrinking cell–cell junction.[58] These studies suggest that actomyosin, the driving force of the contraction mode, has a close functional relationship with E-cadherin.

The contraction mode theoretically requires the dynamic regulation of cell–cell adhesion in the adjacent cells exhibiting cell–cell junction shrinkage and the cells consequently being pulled by this neighboring cell junction (Figure 5(b)). The contraction at the cell–cell junction originates from two independent layers of cell cortices belonging to distinct individual cells. Therefore, these two cells need to adhere to each other to properly coordinate the shrinkage event at the shared junction (i.e., dynamic adhesion, Figure 5(b), green line). Efficient shrinkage at the junction might also be interrupted because of excessively strong cell adhesion. On the other hand, neighboring cells have to adhere more tightly to cells displaying this contracting cell–cell junction to allow a proper inward pull (i.e., a tighter adhesion, Figure 5(b), red line). Indeed, a previous study of germband extension in *D. melanogaster* showed that the contracting cell–cell junction had lower accumulation of β-catenin (known to interact with E-cadherin at adherens junctions) and a higher turnover rate than neighboring cell–cell junctions.[43,59] This suggests that the constricting cell–cell adhesion is persistent but very fluid in the contraction mode. Furthermore, the strong adhesive force must be properly disassembled during junctional remodeling after cell–cell junction shrinkage (Figure 2(b)), and subsequently produce new

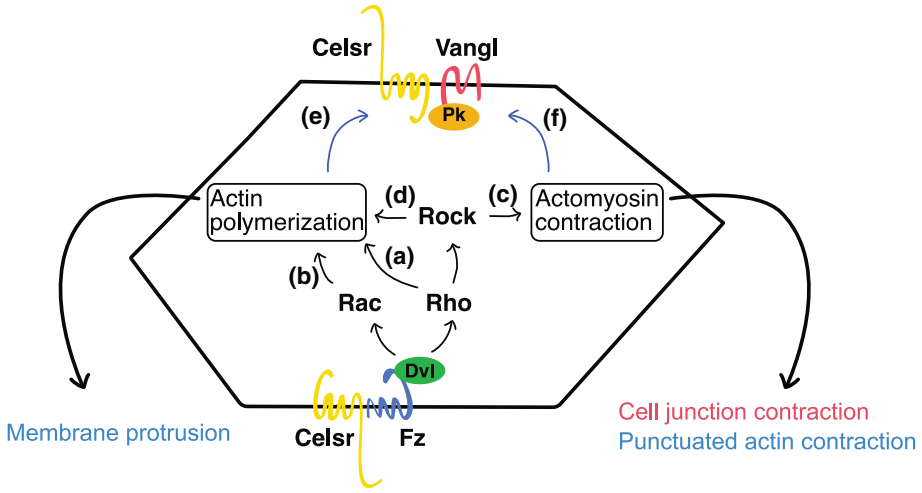

FIGURE 6 | Planar cell polarity (PCP) pathway regulates actin dynamics via small guanosine triphosphatases (GTPases). Downstream pathways of the PCP pathway for cytoskeletal regulations. Activation of (a) actin polymerizing factor Daam1 by Rho, and (b) actin branching factor Arp3 by Rac. (c) Phosphorylation of myosin light chain (Myl) by Rho and Rock (Rho-Rock-Myl cascade). (d) Phosphorylation and activation of LIM-kinase (LIMK) controlling F-actin severing cofilin function by Rho and Rock. (e) A feedback loop of cofilin on PCP proteins. (f) A feedback loop of contractile forces of actomyosin on PCP proteins.

adhesions to reconnect the cell junctions. Such switching of adhesion properties synchronized with junction shrinkage is unique to the contraction mode. Theoretically, this property is not required for the crawling mode as the cells do not need strong and stable adhesion for their movement.

Currently, the adhesion molecules involved in the contraction mode of *X. laevis* notochord formation remain unknown. Adhesion G protein-coupled receptors (GPCRs) are another candidate group of proteins thought to regulate cell adhesion in the contraction mode for CE. Gpr125, a member of the GPCR family, was reported to localize along the cell–cell junction at the animal pole and dorsal mesoderm in zebrafish embryos.[60] Considering its localization at the cell–cell junction, Gpr125 may play a significant role in the regulation of cell adhesion during the contraction mode. In addition, Celsr, another member of the GPCR family and also a planar cell polarity (PCP) protein (introduced in the next section), is suggested to function as a key cell adhesion molecule in the contraction mode of CE according to its observed colocalization along the cell–cell junction with actomyosin.[8] So although there are plenty of candidate mechanisms and molecules for adhesion, the dynamic regulation of adhesion molecules during the contraction mode remains to be elucidated.

Molecular Pathways/Regulators for the Coordination of Cytoskeleton Dynamics and Cell Adhesion in the Crawling and Contraction Modes of CE

Actin, which shows regulated localization in a cell displaying specific polarity, is an essential driving force that enables cell movements in both the crawling and contraction modes. This section discusses the molecules involved in determining tissue polarity, which ultimately regulates cytoskeleton and cell adhesion molecules during CE.

PCP Pathway

Among molecular contributors, factors in the PCP pathway were identified as providing indispensable signaling functions to the process of CE. Numerous studies utilizing tissue explant systems to investigate *X. laevis* notochord development have contributed to the identification of the molecular mechanisms underlying CE. The PCP pathway is composed of both transmembrane proteins such as Frizzled (Fz), Vangl, and Celsr and cytoplasmic proteins such as Dishevelled (Dvl) and Prickle (Pk). The asymmetric intracellular distribution of these PCP proteins is suggestive of their contribution to polarized cell behaviors canonical to CE. Moreover, studies have shown that small GTPases, Rho and its downstream kinase Rho-associated protein kinase (Rock), mediate the signaling originating from PCP components to regulate subsequent cytoskeleton dynamics in *D. melanogaster* and *X. laevis* embryos.[37,61,62] Rho and Rock are central in the molecular cascade located downstream of PCP proteins and known to regulate both actin polymerization and actomyosin contraction (Figure 6).

PCP Pathway in the Crawling Mode

Inhibition of PCP proteins results in failure of notochord cells to build stable bipolar membrane protrusions, leading to the subsequent arrest of tissue elongation during CE. The importance of the PCP pathway for CE was first revealed when one of its active components, Dvl, was purposefully inhibited in the *X. laevis* model. As a result, the typical explant elongation and establishment of cell polarity, including the bipolar membrane protrusions were disrupted.[63,64] The following observations suggest that the PCP pathway influences both the behavior of the cytoskeleton and cell adhesion events in cell crawling during CE.

The inhibition of Dvl in *X. laevis* embryos disrupted actin polymerization by blocking the function of the Formin family protein Daam1 via Rho,[36] and Arp3 via Rac[35] (Figure 6(a) and (b), respectively). This disruption resulted in reduced polarization and branching of actin filaments at the membrane protrusions. As mentioned previously, the crawling mode is driven by both membrane protrusion and actin contraction, and it is thought that phosphorylation of the myosin light chain (Myl) by Rho and Rock may be involved in the actin cable contraction that exerts pulling force[65] (Figure 6(c)). In addition, another cascade is likely involved in the regulation of CE through phosphorylation and activation of LIM-kinase (LIMK) by Rock (Figure 6(d)). LIMK subsequently phosphorylates itself to inhibit the function of an actin-binding protein, Cofilin. Inhibition of Cofilin may be required for the actin filament dynamics at the membrane protrusion to enable cell crawling.[66,67] Indeed, previous studies suggested that Cofilin actually controls the localization of PCP proteins Vangl2 and Celsr[39] (Figure 6(e)) rather than being merely a downstream factor.

The PCP pathway is also closely associated with the cell–cell adhesion and cell–matrix adhesion. As mentioned above, Papc is responsible for the regulation of membrane protrusions during the early phase of CE in *X. laevis* notochord formation.[68,69] The PCP protein Fz7 is required for the proper

localization of Papc at the cell membrane,[69] which in turn acts as a regulator of the dynamic localization of Dvl1[68] (Figure 7). The PCP pathway is also indispensable for the assembly of fibronectin on notochord surface, permitting the cell crawling process presumably via integrin–fibronectin signaling involving focal adhesions during CE in the *X. laevis* notochord development.[54,76] Vice versa, fibronectin alters localization of the PCP protein in the explants.[55]

PCP Pathway in the Contraction Mode

The previously mentioned Rho-Rock-Myl cascade may also be the main signaling pathway regulating the contraction mode of CE in vertebrates (Figure 6(c)). A study of neural plate formation in chick embryos indicated that the PCP protein Celsr is required for the activation of actomyosin at the cell–cell junction.[8] Furthermore, another PCP protein, Dvl, is also required for the localization of actomyosin during both kidney tubule elongation and notochord formation in *X. laevis*.[11] Inhibition of Dvl during *X. laevis* notochord formation[28,37,77] results in the reduction of available phosphorylated Myl, suggesting that the PCP pathway regulates both localization and activation of the driving force during the contraction mode. In addition, recent studies note the possibility of a feedback mechanism in which actomyosin also regulates the distribution of PCP proteins. This is supported by evidence showing that inhibition of actomyosin activation causes a disturbed localization of PCP proteins in the *X. laevis* neural plate[78] and *C. intestinalis* notochord[46] development (Figure 6(f)).

The PCP pathway appears to be responsible for cell adhesion events occurring during the contraction mode of CE. The relationship between PCP proteins and cadherin was reported previously in non-CE processes. Knockdown experiments of PCP proteins Scribble, Fz, or Pk disrupted the turnover rate and localization of cadherin in cultured Madin-Darby canine kidney (MDCK) cells[79]; tracheal branching of *D. melanogaster*[80]; and Kupffer's vesicles of zebrafish embryos.[81] The PCP protein Vangl was also reported to bind to E-cadherin in HEK293 cells *in vivo*, and these two proteins were confirmed to be colocalized in the kidney tissue of rats[70] (Figure 7). The regulations of cell adhesion molecules for the contraction mode are not yet fully understood. Therefore, investigating the roles of cell adhesion molecules that are potentially regulated by the PCP pathway may be of interest.

Non-PCP Regulators: Fibroblast Growth Factors (FGF) and Shroom

The fibroblast growth factors (FGF) signaling pathway is another potentially major upstream regulator of cytoskeletal and adhesion molecules involved in CE that elicits membrane protrusions in the crawling mode. The downstream factors of the FGF signaling pathway have been reported to control membrane protrusions and Papc function in *X. laevis* notochord formation[82] (Figure 7). In addition, Fgf3 is suggested to be one of the regulators of proper localization of Dvl in *C. intestinalis* notochord formation.[71]

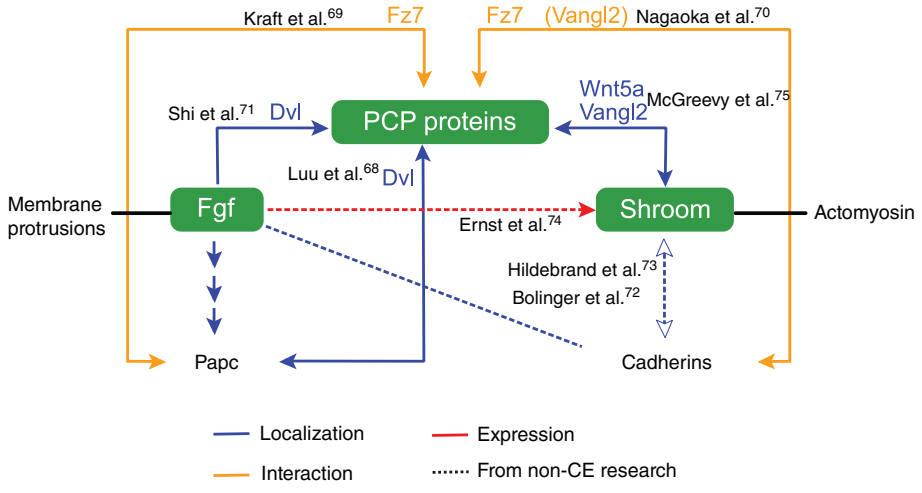

FIGURE 7 | Interplay between the regulators of cytoskeleton components and cell adhesion molecules. Interplay between the planar cell polarity (PCP) pathway, Shroom, and fibroblast growth factors (FGF) for the regulation of downstream cytoskeleton components and cell adhesion molecules. The nature of each relationship is classified by localization (blue line), expression (red line), or physical interaction (yellow line). Solid and dotted lines are indicative of the relationship being demonstrated by studies investigating convergent extension (CE) and non-CE, respectively.

Therefore, the FGF signaling pathway along with the PCP components may affect CE (Figure 7).

Shroom3 is another non-PCP molecule, which may be involved in the regulation of the contraction mode in CE, especially in epithelial tissues. Shroom3 was originally investigated as an essential factor responsible for neural tube closure in mouse embryos.[83] The Shroom protein family is a group of actin-binding proteins required for apical contraction, a major driving force for neural tube closure in *X. laevis* embryos.[84] A study in chick embryos showed that Shroom3 binds to Rock, a core mediator located downstream of the PCP pathway regulating actomyosin activity and influences apical constriction for neural tube closure.[85] In that study, the investigators showed that phosphorylated myosin was unable to localize at the correct cell–cell junction without the presence of Shroom3, suggesting that Shroom3 is an essential factor for the proper execution of the contraction mode. Furthermore, Shroom was shown to colocalize with the structural components of adherens junctions and tight junctions such as E-cadherin, β-catenin, and Zo-1 in the mouse neural ectoderm, *D. melanogaster* embryos and MDCK cells.[72,73] These findings indicate that Shroom may regulate cell adhesion during the contraction mode in epithelial CE. However, Shroom expression is detected exclusively in epithelial tissues in vertebrate embryos,[86] suggesting that the ability of this molecule to elicit a CE response is limited to epithelial tissues in vertebrates.

Germband extension in *D. melanogaster* does not require the PCP pathway, but instead, Shroom may regulate actomyosin contraction in invertebrates. Indeed, Shroom is required for germband extension in *D. melanogaster* embryos.[87] Another potential regulator for the contraction mode in *D. melanogaster* germband is the Toll receptor family, which was reported to direct planar polarity of actomyosin contraction.[88] The reasons for the different use of the PCP pathway by vertebrates, compared to invertebrates, in early embryonic elongation remain unclear.

It is notable that the FGF pathway, Shroom, and PCP pathway influence each other to regulate the cytoskeleton and cell adhesion process during CE (Figure 7). Fgf is known to induce mRNA expression of Shroom during the process of proneuromast assembly in zebrafish embryos.[74] More recently, double knockouts of Shroom3 and a PCP protein (Vangl2 or Wnt5a) in mouse embryos resulted in severe neural tube closure phenotypes such as open spinal cord and short body axis, suggestive of the genetic interaction that exists between Shroom and PCP proteins during vertebrate development.[75] In that double-knockout study, control animals accumulated Shroom along with PCP proteins and actomyosin at cell–cell junctions in the neural tube. This suggested that Shroom coordinates the contraction of these cell–cell junctions together with the PCP pathway during CE (Figure 7). This global interplay between key factors and signaling networks may be the basic mechanism behind the cytoskeletal and cell adhesion behaviors necessary to result in collective cell movement without disrupting tissue integrity.

CURRENT QUESTIONS AND FUTURE CHALLENGES

Other Downstream Machineries for Cell Movement in CE

The mechanism linking the polarized cytoskeletons with cell adhesion molecules during *X. laevis* notochord formation is still under investigation. However, studies in *D. melanogaster* germband extension provide evidence that the distinct behaviors of cell adhesion molecules during CE depends largely on cytoskeleton polarity.[59] Molecules such as Ezrin, Moesin, and Radixin that mediate the interaction between the cytoskeletons and cell adhesion molecules, as well as between the cytoskeletons and the cell membrane, may be part of an important machinery coordinating cell movement with neighboring cells in tissue undergoing morphogenesis[89] Further investigation of the roles of these intracellular proteins will be necessary to understand how the cytoskeletal behaviors link to those in neighboring cells during CE.

The functional interaction between actin and microtubules is also not yet elucidated. Previous *in vitro* studies showed that microtubule filaments were stabilized in a myosin heavy chain-deficient environment, leading to decreased cell contractility and supporting the functional interaction of actomyosin with microtubules.[90] More recent studies demonstrated that myosin phosphatase, a common enzyme facilitating the dephosphorylation of the myosin light chain, inactivates microtubule deacetylase (HDAC6). Consequently, the acetylation of microtubules inhibits focal adhesion disassembly, leading to decrease in cell motility.[91] Another *in vivo* study showed that microtubules are required for actomyosin contractility that leads to apical constriction on the luminal side of salivary gland tubes in *D. melanogaster*[92] and also for wound closure in *Xenopus* oocytes.[93,94] These studies suggest that functional interaction of actomyosin with microtubules may be necessary to execute cell movement.

Interplay Between the Crawling and Contraction Modes During Morphogenesis

Although it is likely that these modes may have adapted to specific features and constraints within different tissues, recent studies suggest that these modes coexist in a single tissue as observed in the mouse neural plate,[9] *X. laevis* notochord,[28] and *D. melanogaster* germband[29] development.

During *X. laevis* notochord formation, each mode may have a different temporal role within the process. Hence, the crawling mode may be predominantly utilized in early gastrulation, whereas the contraction mode may be used later in gastrulation and neurulation. As mentioned previously, the properties of cell adhesion may also be subjected to transformation throughout CE during notochord formation.

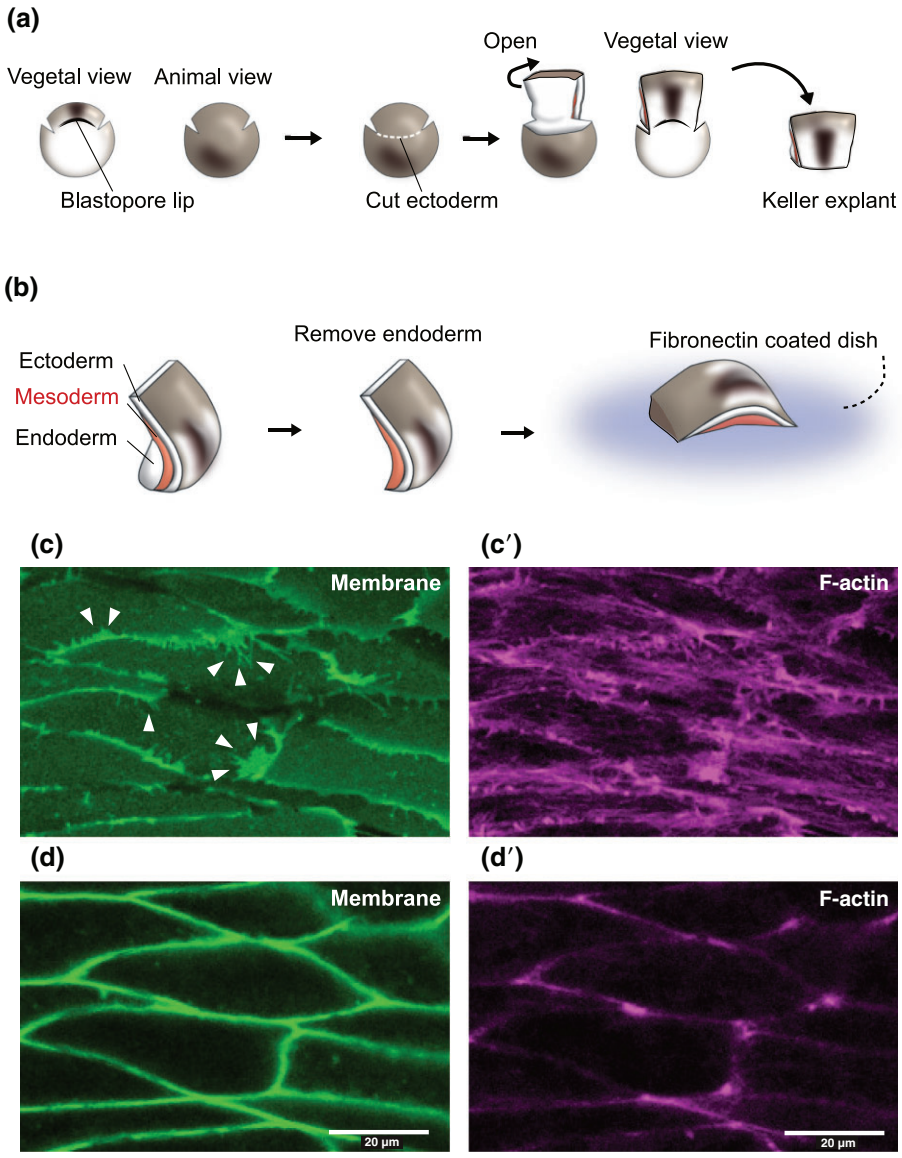

FIGURE 8 | Tissue explant isolation from *Xenopus laevis* embryos for live imaging. (a) Procedure of isolating Keller explants. The explant is cut out at embryonic stage 10.5. Incisions are made on both sides of the blastopore lip, and the dorsal region is opened after cutting the ectoderm. The dorsal region is discerned by cutting along the blastopore lip. (b) Trimming the Keller explant and imaging the notochord. The endoderm is removed to expose the mesoderm (notochord) before mounting on a fibronectin-coated dish. The mesoderm is placed face down for the purpose of live imaging through inverted confocal microscopy. (c–d′) Images of the cell membrane and F-actin captured by live imaging of Keller explants. (c) and (c′) are images of the cells at the surface of the explant, where the cells adhere to the fibronectin. (d) and (d)′ are images of the same cells as in (c) and (c′), but focused 5 μm deep from the plane of (c) and (c′). The membrane protrusions (arrowheads) and actin cables are more obvious in (c) and (c′) images, whereas the borders of the cells are more obvious in (d) and (d′) images.

Thus, it is possible that CE necessitates a combination of multiple driving forces to properly produce a continuous and dynamic developmental process. Furthermore, a specific tissue type may employ one or both modes depending on cell adhesion properties and interactions with other cells or ECM.

Another possible explanation for the existence of two distinct modes of CE in a single tissue is that two different driving forces may need to be utilized to effectively move a complex, three-dimensional cell within the cellular environment. Morphogenesis is a multifaceted process requiring each cell to acquire a specific mobility to achieve this end goal. Such a diverse cell environment may necessitate the use of different forces: for example, the basal side of a cell can exhibit a feature of membrane protrusions (i.e., the crawling mode), whereas the apical side of a cell can exhibit contraction of cell–cell junctions (i.e., the contraction mode), as the study of *D. melanogaster* germband proved very recently.[29] Intriguingly, in CE during *X. laevis* notochord formation, membrane protrusions and punctuated actin contraction, two distinct features of the crawling mode, are plainly observed at the surface of the Keller explants (Figure 8(b), (c), and (c′)). On the other hand, actomyosin at the cell–cell junction—evidence of the contraction mode—is detected at approximately 5 μm deep the plane of membrane protrusion (Figure 8(d) and (d′)). The mode of interaction between these distinct driving forces within a cell remains unknown, mainly because of the technical limitations on three-dimensional live imaging of whole tissue *in vivo* during CE. Future advances in imaging may overcome this technical limitation and allow investigators to further explore the biological significance of the two distinct modes of CE.

The contraction mode appears to be the one broadly conserved in a variety of tissues from invertebrates to vertebrates. However, it is also proposed that the crawling mode functions together with the contraction mode in many contexts. Currently, the hypothesis that crawling and contraction modes work together has only been tested in a handful of tissue types. In addition to these two modes, one may also consider the possible existence of other unknown CE modes, because studies have observed external physical forces from neighboring tissues. For example, neighboring tissues affect CE in notochord and cartilage elongation in vertebrates,[95–97] as well as in invertebrate germband extension.[98–100] The synergistic action of the local force in a cell (i.e., membrane protrusions and junction contraction) and tissue-scale forces could achieve the efficient coordination of CE.

SUMMARY

This review discusses two modes of CE, a conserved collective cell rearrangement taking place during morphogenesis. The similarities and differences in the source and mechanism behind the driving forces of cell movement between these two modes were highlighted. Each mode involves a specific set of associated cytoskeletal behaviors, cell adhesion molecule functions, and molecular signaling, yet they both accomplish the same developmental goal. The coordination of multiple cell movement during CE remains partially unexplained, however, it is clear that complex, multiple molecular mechanisms determining cytoskeletal and cell adhesion behaviors are required for CE.

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