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Transgenic Techniques for Investigating Cell Biology During Development

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Abstract

Ascidians are increasingly being used as a system for investigating cell biology during development. The extreme genetic and cellular simplicity of ascidian embryos in combination with superior experimental tractability make this an ideal system for in vivo analysis of dynamic cellular processes. Transgenic approaches to cellular and sub-cellular analysis of ascidian development have begun to yield new insights into the mechanisms regulating developmental signaling and morpho-genesis. This chapter focuses on the targeted expression of fusion proteins in ascidian embryos and how this technique is being deployed to garner new insights into the cell biology of development.

Keywords

Ascidians; *Ciona intestinalis*; *Phallusia mammillata*; *Halocynthia roretzi*; Cardiac induction; Collective cell migration; Cell cycle progression; Spindle positioning; Fusion proteins

14.1 The Study of Developmental Cell Biology in Ascidians

The development of multicellular organisms requires the precise coordination of processes within and between cells. However, high- resolution in vivo analysis of the coordinated cellular processes underlying development has been hindered by large cell numbers and technical challenges associated with manipulating and monitoring cells in developmental model systems. Ex-vivo cell culture systems have allowed researchers to circumvent some of these challenges, reducing cellular complexity and facilitating in vivo imaging. Yet, ex-vivo models fail to recapitulate the diversity of cellular interactions present in animal systems.

Ascidians have emerged as powerful systems for the in vivo analysis of cellular mechanisms underlying development. Development of transparent ascidian embryos is rapid. Larval development proceeds through a well-characterized series of stereotypic cell divisions that culminate within ~12 h post-fertilization. At the end of this period, each transparent free-swimming ascidian larva consists of fewer than 3,000 cells (Satoh 1994). In addition to their extreme cellular simplicity, ascidian genomes are compact, which greatly facilitates the identification of *cis*-regulatory elements controlling gene expression (Satoh et al. 2003).

Moreover, ascidian genomes contain single orthologs to most vertebrate genes (Dehal 1542002). This comes in stark contrast to vertebrate genomes, which possess multiple paralogous copies of genes as a result of genome duplication. Together, the extreme cellular and genetic simplicity of ascidian embryos greatly facilitates high resolution in vivo analysis of intracellular dynamics driving critical signaling and transcriptional decisions during development.

Ascidian embryos are particularly amenable to transgenic manipulations. Established injection and electroporation protocols allow researchers to rapidly and reliably generate thousands of transgenic embryos for experimental analysis (Hikosaka et al. 1993; Corbo et al. 1997; Zeller 2004). The ease and efficiency of generating transient transgenic embryos greatly facilitates high-throughput in vivo analysis of cellular processes. Genes of interest may be expressed in embryos ubiquitously or under the control of well-characterized, lineage-specific drivers (*cis*- regulatory enhancer + promoter) (Stolfi and Christiaen 2012). Transgenically expressed proteins can be used to monitor gene expression, label cells and organelles, or perturb gene function.

A wide variety of transgenic approaches to manipulating gene function have been adapted for use in ascidian embryos. Classically, these transgenic manipulations of gene function have relied heavily on the expression of constitutively active or dominant negative constructs (Stolfi and Christiaen 2012). More recently, however, knockdown and genome editing technologies have been adapted for use in ascidian systems, including: RNA interference (RNAi) (Nishiyama and Fujiwara 2008), zinc finger nucleases (ZFNs) (Kawai et al. 2012), transcription activator-like effector nucleases (TALENs) (Treen et al. 2014; Yoshida et al. 2014) and most recently clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) (Sasaki et al. 2014; Stolfi et al. 2014). The application of techniques in ascidian embryos has already been surveyed in the opening chapters of this volume. Thus, this chapter focuses specifically on transgenic tools used to visualize and perturb fundamental cellular processes in ascidian embryos.

The adaptation of fluorescent proteins for use in ascidians has revolutionized the study of cellular processes in these animals. In the two decades that have passed since it was first demonstrated that heterologous expression of green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* could be used as a non-invasive means of monitoring gene expression in vivo (Chalfie et al. 1994), strategies utilizing fluorescent proteins have been developed to visualize subcellular structures and organelles, visualize protein interactions, and monitor the distribution and trafficking of protein in cells (Miyawaki 2011). The relative ease of generating transgenic ascidian embryos coupled with their developmental robustness towards large quantities of exogenous DNA facilitates the expression of fluorescent proteins in ascidian embryos. Fluorescent proteins may be expressed as standalone proteins or as chimeric fusion proteins, in which the sequence encoding the fluorophore has been fused to a sequence encoding a protein of interest. The later approach represents a powerful strategy for monitoring cellular processes in vivo. Moreover, multiple fusion proteins can be expressed simultaneously in a single embryo to concomitantly label multiple cell populations or subcellular structures. Co-expression of fusion proteins can be used to assay the relative distribution of proteins (co-localization; Denker et al. 2013; Sehring et al. 2014;

Gline et al. 2015; Newman-Smith et al. 2015) or identify protein interactions (fluorescence resonance energy transfer; Cooley et al. 2011). Additionally, both free fluorophores and fusion proteins can be expressed in embryos in conjunction with perturbation constructs (e.g., dominant-negative forms, CRISPR/Cas9 gene-targeting plasmids) to assess the impacts of these constructs on a cell or process of interest (Stolfi and Christiaen 2012).

14.2 Codon Optimization of Sequences Encoding Fluorescent Proteins

Commercially available fluorescent protein sequences have been widely used by ascidian biologists for visualizing cells and proteins in developing embryos. These fluorescent proteins are easily obtained and available with a range of spectral properties (Day and Davidson 2009). Moreover, sequences encoding many commercially available fluorescent proteins have been modified to improve their photostability, increase the rate of protein maturation and folding, or prevent oligomerization of fluorophores in cells (Shaner et al. 2004; Zeller et al. 2006; Shaner et al. 2008). The sequences encoding several commonly used, commercially available fluorescent proteins (e.g., eGFP, mCherry) have also been human codon-optimized to improve the efficiency of translation in mammalian cells (Yang et al. 1996; Snapp 2009). Despite having been selected for optimal performance in mammalian cells, even the human codon-optimized fluorescent proteins can be readily expressed and detected in ascidian systems (Zeller et al. 2006; Passamaneck et al. 2006). However, Zeller et al. demonstrated that commercially available eGFP, codon-optimized for expression in human cells, is sub-optimal for use in *Ciona intestinalis* embryos. Indeed, they showed that codon-optimized eGFP took longer to reach detectable levels in *Ciona* embryos than wild-type *Aequorea*-derived GFP. The Zeller group therefore generated a set of fluorescent protein sequences that have been codon-optimized for use in *Ciona* (green [GFPci], cyan [CFPci], yellow [YFPci], red [RFPci]; http://www.bio.sdsu.edu/faculty/zeller/Vector_Sequences.html) (Zeller et al. 2006). The *Ciona* codon-optimized, GFP-derived fluorescent proteins may be detected as many as 90 min earlier than their unoptimized counterparts (CFPci vs CFP) and may result in ~50% increase in detectable fluorescence (GFPci vs GFP) making them ideal for experiments that require robust, early fluorescence (Zeller et al. 2006).

14.3 Fusion Proteins for Visualization of Cellular Processes in Ascidian Embryos

Fluorophore-tagged fusion proteins enable in vivo analysis of cellular proteins, structures, and organelles. Transgenic embryos, both live and fixed, can be imaged with sub-cellular resolution using standard confocal microscopy (Negishi et al. 2013; McDougall et al. 2015). A growing collection of fluorescent protein fusions (FP fusions) have been generated that label specific cellular structures or compartments in ascidian embryos (Table 14.1). High-resolution in vivo analysis of the spatiotemporal distribution of fusion proteins has provided mechanistic insight into a wide range of dynamic cellular processes. In the following sections, we discuss several examples, including cell division, spindle-positioning, morphogenesis, and cell signaling.

14.3.1 Cell Cycle Progression

Fusion proteins have been employed extensively to monitor the spatial and temporal dynamics of cell-cycle progression in ascidian embryos. Activation of the zygotic genome is strongly associated with shifts in the duration of cell-cycle phases. Dumollard et al. generated transgenic *Phallusia mammillata* embryos expressing a fluorophore-tagged histone, H2B::mRFP, to examine cell-cycle progression during zygotic genome activation in ascidians (Dumollard et al. 2013). Fluorophore-tagged histones are incorporated into DNA-bound nucleosomes, efficiently labeling chromatin in both interphase and mitotic cells and providing an efficient way of monitoring cell-cycle progression in vivo (Kanda et al. 1998). Using this approach, they were able to identify two distinct cell cycle phases in early ascidian embryos modulated by β -catenin-dependent transcription. Intriguingly, β -catenin-dependent activation of the zygotic endomesodermal gene regulatory network at the 16-cell stage was associated with a shortened interphase. In contrast, subsequent β -catenin-dependent transcription in the endoderm just prior to gastrulation was associated with prolonged interphase (Dumollard et al. 2013). These results highlighted specific regulatory circuits capable of modifying cell-cycle progression. This study also suggested that modulation of cell-cycle progression in the early embryo might be driven by transcriptional programs associated with cell fate induction.

Transgenically expressed fluorescence ubiquitination cell cycle indicator (FUCCI) probes represent an alternative method of monitoring cell-cycle phasing in living cells using a two-color fusion protein system (Sakaue-Sawano et al. 2008). Labelled Cdt1 protein with a peak expression during G1 and its inhibitor, geminin, whose own expression peaks during S and G2 phases, are simultaneously expressed in cells. Time-lapse imaging is then used to monitor the fluorescence of the two FUCCI probes and thereby monitor cell cycle progression. The simultaneous expression of these two probes enables more precise discrimination between cells in G1 and S/G2/M phases than is possible relying only on markers of chromatin confirmation alone (Sakaue-Sawano et al. 2008). Ogura et al. employed FUCCI technology to examine cell-cycle progression in the epidermis of *Ciona intestinalis* embryos during neurulation. Using this approach, they were able to identify a prolonged G2 phase in epidermal cells associated with neural tube closure/epidermal fusion. They were also able to demonstrate that precocious division of the epidermis resulting from ectopic expression of the cell cycle regulator, Cdc25, disrupted neural tube closure. In addition, live imaging of the epidermal divisions revealed that resumption of cell cycle progression is staggered along the anterior-posterior (AP) axis, such that cells posterior to the embryo divide before those anterior. The staggered division of epidermal cells correlate with AP zippering of the neural tube. Epidermal division that occurs at this time is oriented along the AP axis contributing to elongation of the embryo along this axis (Ogura et al. 2011).

14.3.2 Spindle Positioning

Imaging of transgenic ascidian embryos has also garnered insights into the mechanisms driving spindle positioning in the early embryo. Ascidian embryos display a unique invariant cleavage pattern prior to gastrulation that is dependent upon the precise orientation of the cell division plane, including some instances of unequal cleavage. Work in *Halocynthia*

roretzi embryos initially showed that the orientation and positioning of the cleavage plane during these unequal cleavages is mediated by an electron-dense, multilayered structure termed the centrosome-attracting body (CAB) (Hibino et al. 1998; Nishikata et al. 1999; Iseto and Nishida 1999). Subsequent work has shown the CAB to be highly conserved amongst ascidian species (Brown and Swalla 2007; Hibino et al. 1998; Nishikata et al. 1999; Iseto and Nishida 1999; Sardet et al. 2003; Patalano et al. 2006; Gyoja 2006). Prodon et al. employed 4D live imaging to examine CAB-mediated spindle dynamics during unequal cleavage in transgenic *Phallusia mammillata* embryos expressing a GFP-tagged microtubule binding protein (MAP7::EGFP). During the interphase, the nucleus and associated centrosomes migrate toward the CAB. As the spindle begins to form during mitotic entry, the nearest, medial spindle pole is attracted to the CAB, where it remains tethered through cytokinesis. Finally, tethering of the lateral spindle pole further rotates the mitotic spindle during anaphase. Blastomere isolation experiments demonstrated that tethering is dependent upon cell–cell contacts. Further analysis of furrow formation following orientation of the mitotic spindle in transgenic embryos expressing either a membrane-tethered GFP (PH::EGFP) or a Venus-tagged actin-binding domain (ABD::Venus) revealed an unusual process of asymmetric furrow formation in these cells during cytokinesis. In particular, lateral spindle attachment to cell–cell contact appears to inhibit initiation of the cytokinetic furrow at the site of attachment (Prodon et al. 2010).

14.3.3 Morphogenesis of the Ascidian Neural Tube

The ascidian neural tube arises from a flat sheet of ~40 neural progenitor cells termed the neural plate. Primary invagination of the neural plate occurs during gastrulation. (Nicol and Meinertzhagen 1988b; Nicol and Meinertzhagen 1988a). Live imaging of primary neural plate invagination in transgenic embryos expressing H2B::YFP revealed a two-step process in which the medial cells of the neural plate undergo a single oriented division aligning the daughter cells along the AP axis. These cells then intercalate via a convergent extension to form the ventral floor-plate of the neural tube, a single linear row of cells extending along the AP axis of the embryo. Subsequently, the lateral cells of the neural plate undergo concomitant lateral stacking, forming two lateral rows of cells flanking the ventral floorplate. These cellular rearrangements are dependent on fibroblast growth factor (FGF) and nodal signaling, respectively (Navarrete and Levine 2016).

A subsequent secondary invagination completes the closure of the neural tube. Secondary invagination of the posterior neural tube occurs via unidirectional zippering, which proceeds in a posterior to anterior direction and completes formation of the nerve chord. The cellular mechanism underlying neuroepidermal zippering was recently described by Hashimoto et al. (2015). Using transgenic embryos expressing a GFP-labeled marker of tight junctions (ZO1::3×GFP) in the neural plate and overlying epidermis in conjunction with live imaging, they observed a striking pattern of junctional contractions occurring ahead of the closing “zipper” of the neural tube. Using an embryo expressing a YFP-conjugated intrabody to monitor activated myosin (iMYO::YFP), they found that junctional contractions were associated with a transient increase in activated myosin. Further investigation of the actomyosin contractions during this process using a combination of transgenic manipulations and cytoskeletal inhibitors indicated that myosin is activated in cells by

ROCK/Rho. Finally, laser ablation of junctions revealed that the transient activation of myosin ahead of zippering generated increased the tension across tight junctions required to pull the neural plate closed at the midline. Junctional exchange ahead of cell detachment serves to dissipate this increased tension and promote zipper progression.

14.3.4 Cell Migration

The migration of two bilaterally positioned pairs of cardiopharyngeal progenitors (trunk ventral cells; TVCs) during development of the ascidian heart represents an elegant, highly simplified model for investigating collective cell migration. TVCs migrate as polarized pairs of cells, tethered to one another by cadherin-rich cell–cell adhesions (Norton et al. 2013; Gline et al. 2015). High-resolution analysis of transgenic TVCs expressing a GFP-tagged moesin fusion protein (GFP::Moesin) revealed a pronounced leader–trailer polarity, in which the leader cells exhibit robust enrichment of lateral actin-rich protrusions at the leading edge, whereas the trailer cell displays a concomitant constriction of the trailing edge (Christiaen et al. 2008; Gline et al. 2015).

Analysis of transgenic TVCs in which mosaic incorporation of transgenes resulted in the selective inhibition of cell migration in either the leader or the trailer revealed that individual TVCs are capable of migrating alone. However, using the green-to-red photoconvertible fluorophore, Kaede (*Mesp>nls::Kaede*), to label individual cardiopharyngeal founder cells and thereby distinguish between individual transgenic TVCs within pairs, Gline et al. (2015) found that the more anterior founder cell gave birth to the leader TVC in 95% of embryos examined. These results indicated that there is a strong positional bias that contributes to the establishment of leader trailer polarity.

Although TVC pairs are capable of migrating independently, robust migration is dependent on responses to signals from surrounding tissues. The precise extrinsic cues necessary for robust directional migration of TVC pairs have not been identified. However, using live imaging to assess TVC migration (*Mesp>GFP*, *FoxF>H2B::mCherry*), it was shown that extrinsic cues have an impact on migration by altering leader–trailer cell polarity.

By selectively inhibiting secretion in tissues surrounding the TVCs during migration via targeted expression of a dominant negative form of the GTPase, Sar1, in the mesenchyme (*Twist>Sar1^{T35N}*), the notochord (*Brachyury> Sar1^{T35N}*), the epidermis (*EphrinB>Sar1^{T35N}*), and the endoderm (*Nkx2-1>Sar1^{T35N}*), Gline et al. found that polarization of protrusions within the leader–trailer cell pair requires secretion from the endoderm. In contrast, cues secreted from the mesenchyme are required to reinforce trailer cell identity, whereas cues secreted by the epidermis are required to maintain adhesion between leader and trailer cells (Gline et al. 2015).

14.3.5 Signal Processing

The cardiopharyngeal lineage in *Ciona* can be traced back to a multipotent pre-cardiac founder cell lineage established through exclusive expression of the bHLH transcription factor, Mesp (Satou 2004). At the onset of neurulation, each founder cell divides asymmetrically to produce a larger anterior tail muscle cell and a smaller heart progenitor cell (TVC). FGF activates mitogen activated protein kinase signaling (MAPK) in the TVCs,

initiating a well-characterized transcriptional program that induces heart progenitor fate (Evans-Anderson and Christiaen 2016; Cota et al. 2017). During heart progenitor induction, founder cells are exposed uniformly to FGF (Cooley et al. 2011). Analysis of dividing founder cells with transgenic labeling of membrane (GPI::RFP) and filamentous actin enrichment (utrophin::GFP) in transgenic embryos revealed polarized actin- enriched membrane protrusions that correlated with localized inductive signaling along the nascent TVC membrane. Manipulations of actin dynamics including targeted expression of a constitutively active form of CDC42 (CDC42^{Q61L}), co-expression of a truncated form of a downstream actin nucleator (Wasp^{VCA}), along with pharmacological inhibition of actin-related protein (ARP)-mediated actin nucleation (Ck-666), indicated that localized protrusion was required for localized heart progenitor induction (Cooley et al. 2011).

A subsequent study suggested that membrane protrusion drives polarized founder cell adhesion to the underlying extracellular matrix during heart progenitor induction (Norton et al. 2013). Analysis of focal adhesion dynamics in transgenic founder cells expressing GFP::Talin revealed that matrix adhesion is maintained along the presumptive TVC membrane during founder cell division. Targeted perturbations of adhesion through manipulations of Rap GTPase activity and co-expression of specific Integrin receptor chains (Rap^{S17N}, Rap^{G12V} or RapGAP, Integrin- β 1, Integrin- β 2, Integrin- α 11) revealed that localized mitotic adhesion is both necessary and sufficient for polarized induction (Norton et al. 2013).

The most recent study that looked at pre-cardiac founder cells revealed that transgenically labeled FGF receptor (FGFR::Venus) is localized to the adherent membrane during founder cell division. This study also suggested that FGFRs are trafficked in adherent membrane domains enriched with Caveolin (caveolin::GFP/RFP). Targeted disruption and restoration of these adhesive domains using dominant negative constructs (Caveolin^{P132L}, Rap^{S17N}), CRISPR knockdown (*Mesp*>nls::Cas9:nls, *U6*>CavA.39gRNA), and targeted co-expression of Integrin β -chains (*Mesp*>Integrin- β 2 or *Mesp*>Integrin- β 1) showed that they are both necessary and sufficient for polarized FGFR trafficking and during founder cell mitosis and polarized TVC induction immediately after division (Cota and Davidson 2015).

Taken together, these results suggest that polarized adhesive interactions might be able to alter mitotic membrane trafficking. In this way, matrix adhesion can compartmentalize the cellular response to widespread inductive FGF signals.

14.4 Conclusions

Over the past decade, ascidians have emerged as a powerful model in which to study key aspects of cell biology. For this purpose, researchers have adapted a set of molecular tools for labeling cells and intracellular structures that can be used in conjunction with established methods of manipulating gene expression and function to explore the contribution of dynamic cellular processes to development (Table 14.1). However, the repertoire of fusion proteins available for labeling cellular structures in transgenic ascidians remains limited in comparison to more established model systems. Thus, further adaptation of this system requires development of additional cellular reporters.

Most notably absent from the ascidian “tool-kit” are markers of specific membrane compartments within the endolysosomal system, which are critical for future analysis of protein trafficking and degradation in cells. Continuing efforts are also focused on further optimizing protocols for live imaging. Although high-resolution live imaging has been successfully employed to examine cellular processes in early ascidian embryos (Ogura et al. 2011; Dumollard et al. 2013; Negishi and Yasuo 2015), the success of this approach has been limited at later stages of development, particularly in cells positioned deep within embryos.

The continued development of improved methods for transgenically manipulating the optically transparent ascidian, *Phallusia mammilata*, holds great promise in overcoming this limitation (Negishi et al. 2013; McDougall et al. 2014; McDougall et al. 2015). Moreover, newly developed SNAP, CLIP, and HALO peptide-tagging strategies that allow covalent chemical labeling of proteins in vivo hold some potential for achieving brighter, more photo-resistant labeling of proteins to facilitate live imaging (Fig. 14.1) (Keppler et al. 2003; Gautier et al. 2008; England et al. 2015).

Despite these challenges, research into this system has already begun to provide novel insights into the cellular mechanisms underlying development. In particular, ascidian researchers have begun to integrate transgenic approaches to in vivo analysis of cellular processes with existing methods for manipulating gene function. This integrative approach to investigating cellular processes during ascidian development has proven to be a powerful strategy for identifying the molecular mechanisms underlying complex developmental processes, including morphogenesis, cell migration, and cell signaling.

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Fig. 14.1.

CLIP, SNAP, and HALO tags for covalent labeling of ascidian protein. Genetically encoded CLIP tag (New England BioLabs, NEB), SNAP tag (NEB) and HALO tag (Promega) schematically depicted in **(a)** can be used in conjunction with commercially available fluorophore-conjugated substrates to covalently label and visualize proteins in living or fixed transgenic ascidian embryos. **(b)** Ventral projection of a pair of mitotic pre-cardiac founder cells in a *Ciona* embryo expressing an endosome-associated CLIP tag fusion protein under the control of the cardiopharyngeal lineage specific promoter, *Mesp* (*Mesp>CLIP::Rab4*). The embryo was fixed and then incubated with the CLIP-Cell TMR-Star (red; NEB) substrate before imaging using confocal microscopy. Phalloidin was used to detect F-actin and DRAQ5 was used to detect DNA (*blue*). Anterior is to the right

Table 14.1

Ascidian cell imaging tool kit

Structure/molecule	Notes	Species ^a	Promoters	References
Cytoskeletal				
<i>Microtubules</i>				
Ensconsin (MAP7)::3×GFP/RFP	Full-length human E-Map-115 (Ensconsin; GenBank: LT734165.1)	<i>Ci</i>	FOG	Roure et al. (2007)
			Brachyury	Dong et al. (2011)
			Mesp	Cooley et al. (2011)
MAP7::eGFP/RFP/mCherry	Mouse MAP7 N terminal fragment (GenBank: BC052637.1)	<i>Pm</i>		Prodon et al. (2010)
Ensconsin::3×GFP	Mouse MAP7 N terminal fragment (GenBank: BC052637.1)	<i>Pm</i>		Negishi and Yasuo (2015)
<i>Microtubule-binding</i>				
EB3::GFP	Mouse EB3	<i>Pm</i>		Negishi and Yasuo (2015)
<i>Actin</i>				
GFP/mCherry::hActin	Full-length human β -actin	<i>Ci</i>	Brachyury	Dong et al. (2011)
LifeAct::meGFP	First 17 aa of Abp140 (<i>Saccharomyces cerevisiae</i>)	<i>Ci</i>	Brachyury	Dong et al. (2011) and Sehring et al. (2014)
LifeAct::GFP/RFP	First 17 aa of Abp140 (<i>Saccharomyces cerevisiae</i>)	<i>Pm</i>		McDougall et al. (2015)
mCherry::UtrCH	CH domain from <i>Ciona</i> utrophin	<i>Ci</i>	Brachyury	Dong et al. (2011)
Utrophin::GFP	First 783 bp of human utrophin (GenBank: NM_007124)	<i>Ci</i>	Mesp	Cooley et al. (2011)
ABD::Venus	ABD from ABP-120	<i>Pm</i>		Prodon et al. (2010)
<i>Actin-binding</i>				
Cofilin::mCherry	<i>Ciona</i>	<i>Ci</i>	FOG	Sehring et al. (2014)
α -actinin::mCherry	<i>Ciona</i>	<i>Ci</i>	FOG	Sehring et al. (2014)
mCherry::tropomyosin	<i>Ciona</i>	<i>Ci</i>	FOG	Sehring et al. (2014)
EB1::mCherry	<i>Ciona</i>	<i>Ci</i>	FOG	Sehring et al. (2014)
IQGAP::mCherry	<i>Ciona</i>	<i>Ci</i>	FOG	Sehring et al. (2014)
Anillin::mCherry	<i>Ciona</i>	<i>Ci</i>	FOG	Sehring et al. (2014)
Septin-2::mCherry	<i>Ciona</i>	<i>Ci</i>	FOG	Sehring et al. (2014)
<i>Myosin</i>				
mCherry::MLC	<i>Ciona</i> MLC	<i>Ci</i>	Brachyury	Dong et al. (2011)
iMyo::YFP ^b	YFP-tagged intrabody that recognizes nonmuscle myosin II A	<i>Ci</i>	FOG	Hashimoto et al. (2015)
Chromatin				
H2B::Venus/CFP/mCherry/RFP/YFP	<i>Human histone 2B</i> (GenBank: X00088)	<i>Ci</i>	FOG	Roure et al. (2007)
			Mesp	Stolfi et al. (2010)
			Zip	Kawai et al. (2015)

Structure/molecule	Notes	Species ^a	Promoters	References
			Tensin	Kourakis et al. (2014)
			Brachyury	Kourakis et al. (2014)
			FoxB	Imai et al. (2009)
			FoxF	Gline et al. (2015)
			Snail	Imai et al. (2009)
			Etr	Veeman et al. (2010)
			Mnx	Navarrete and Levine (2016)
H2B::RFP1	Mouse histone H2B (HH2B) from lung tumor-BI105582)	<i>Pm</i>		Prodon et al. (2010)
Plasma membrane				
GAP43::Venus/CFP	<i>Mouse Gap-43</i>	<i>Ci</i>	FOG	Roure et al. (2007)
			Epi1	Ogura et al. (2011)
GAP43::GFP	First 20 amino acids of <i>Ciona</i> GAP-43	<i>Ci</i>	Bra	Dong et al. (2009)
PH::eGFP/Tomato	PIP2 binding domain from human PLCδ1	<i>Pm</i>		Prodon et al. (2010)
YFP/Kaede/Venus/mCherry::CAAX		<i>Ci</i>	Epi1	Dong et al. (2009)
			Zip	Kawai et al. (2015)
			FoxB	Navarrete and Levine (2016)
memb::GFP	First 20aa of <i>Ciona</i> GAP-43	<i>Ci</i>	Brachyury	Dong et al. (2009)
			Mesp	Cooley et al. (2011)
Caveolin::GFP/RFP	<i>Full-length Ciona</i> caveolin (<i>CavA</i> ; Ciinte.g00012490)	<i>Ci</i>	Mesp	Cota and Davidson (2015)
Endosomes				
Caveolin::GFP/RFP	<i>Full-length Ciona</i> caveolin (<i>CavA</i> ; Ciinte.g00012490)	<i>Ci</i>	Mesp	Cota and Davidson (2015)
Adhesions				
DE-cadherin::mCherry	Open reading frame and the 3' untranslated region for <i>Drosophila melanogaster</i> E-cadherin	<i>Ci</i>	FOG	Roure et al. (2007)
			Brachyury	Dong et al. (2009)
ZO1::3×GFP/tGFP	<i>Ciona</i> ZO-1 (Ci035p23)	<i>Ci</i>	FOG	Hashimoto et al. (2015)
				Denker et al. (2013)
		<i>Pm</i>		Sherrard et al. (2010)
GFP::Talin	<i>Ciona</i> talin (Ciinte.g00005295)	<i>Ci</i>	Mesp	Norton et al. (2013)
Golgi				
ST::GFP	Membrane signal anchor sequence from Rat sialyltransferase	<i>Ci</i>	EpiB	Zeller et al. (2006)
Endoplasmic reticulum				
KDEL::GFP/RFP	<i>Ciona</i> KDEL (ci0100149851)	<i>Ci</i>	Brachyury	Oda-Ishii et al. (2010)
			Mesp	Gline et al. (2015)
Centrosomes/spindle poles			Mesp	

Structure/molecule	Notes	Species ^a	Promoters	References
Aurora::Venus/mCherry	Full-length <i>Ciona</i> aurora kinase (Aur; Ciinte.g00008046)	<i>Ci</i>	FOG	Roure et al. (2007)
	Full-length <i>Ciona</i> aurora kinase (Ciinte.g00008046; transcript model KH.C7.219.v1.A.SL1–1)	<i>Pm</i>		Hebras and McDougall (2012)
Venus/mCherry::First 632 amino acids of Girdin	First 632 amino acids of <i>Ciona</i> Girdin (Ciinte.g00002344; gene model KH2012:KH.C11.506)	<i>Pm</i>	FOG	McDougall et al. (2015)
Plk1::mCherry/Venus	Full-length <i>Ciona</i> PLK1 (Ciinte.g00002774; transcript model KH.C12.238.v1.A.SL1–1)	<i>Pm</i>	Brachyury	Hebras and McDougall (2012)
Midbody				
Aurora::Venus/mCherry	Full-length <i>Ciona</i> aurora kinase (Ciinte.g00008046; transcript model KH.C7.219.v1.A.SL1–1)	<i>Pm</i>		Hebras and McDougall (2012)
Venus:TPX2	Full-length <i>Ciona</i> Tpx2 (Ciinte.g00005340; transcript model KH.C3.181.v1.A.SL1–1)	<i>Pm</i>		Hebras and McDougall (2012)
Incep:mCherry	Full-length <i>Ciona</i> INCEP (Ciinte.g00004061)	<i>Pm</i>		Hebras and McDougall (2012)
Plk1:mCherry/Venus	Full-length <i>Ciona</i> PLK1 (Ciinte.g00002774; transcript model KH.C12.238.v1.A.SL1–1)	<i>Pm</i>		Hebras and McDougall (2012)

Fluorescent fusion proteins and bioprobes used to visualize subcellular dynamics in ascidian embryos (adapted from Negishi et al. 2013; McDougall et al. 2015). *Ciona intestinalis* (*Ci*) and *Phallusia mammillata* (*Pm*). Species, gene model, transcript model, and/or GenBank identifiers for source sequences are indicated where reported

CH calponin homology, *ABD* actin-binding domain, *MLC* myosin II light chain, *YFP* yellow fluorescent protein

^a“Species” indicates species of ascidian in which a probe was used

^b_iMyo:YFP is an intrabody that has been used to recognize activated myosin in *Ciona intestinalis* (Hashimoto et al. 2015)