



HAL
open science

Cell Cycle in Ascidian Eggs and Embryos

Alex Mcdougall, Janet Chenevert, Karen W Lee, Celine Hebras, Remi Dumollard

► **To cite this version:**

Alex Mcdougall, Janet Chenevert, Karen W Lee, Celine Hebras, Remi Dumollard. Cell Cycle in Ascidian Eggs and Embryos. Cell Cycle in Ascidian Eggs and Embryos., pp.153-169, 2011, 10.1007/978-3-642-19065-0_8 . hal-03026008

HAL Id: hal-03026008

<https://hal.science/hal-03026008>

Submitted on 16 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Chapter 8

Cell Cycle in Ascidian Eggs and Embryos

Alex McDougall, Janet Chenevert, Karen W. Lee, Celine Hebras,
and Remi Dumollard

Abstract In ascidians the cell cycle machinery has been studied mainly in oocytes while ascidian embryos have been used to dissect the mechanism that controls asymmetric cell division (ACD). Here we overview the most specific and often exceptional points and events in cell cycle control in ascidian oocytes and early embryos. Mature stage IV eggs are arrested at metaphase I due to cytotstatic factor (CSF). In vertebrates, unfertilized eggs are arrested at metaphase II by CSF. Meta II-CSF is mediated by the Mos/MEK/MAPK/Erp1 pathway, which inhibits the ubiquitin ligase APC/C^{cdc20} preventing cyclin B destruction thus stabilizing MPF activity. CSF is inactivated by the fertilization Ca²⁺ transient that stimulates the destruction of Erp1 thus releasing APC/C^{cdc20} from inhibition. Although many of the components of CSF are conserved between the ascidian and the vertebrates, the lack of Erp1 in the ascidians (and indeed other invertebrates) is notable since the Mos/MAPK pathway nonetheless mediates Meta I-CSF. Moreover, since the fertilization Ca²⁺ transient targets Erp1, it is not clear how the sperm-triggered Ca²⁺ transient in ascidians (and again other invertebrates) stimulates cyclin B destruction in the absence of Erp1. Nonetheless, like mammalian eggs, sperm trigger a series of Ca²⁺ oscillations that increases the rate of cyclin B destruction and the subsequent loss of MAPK activity leading to meiotic exit in ascidians. Positive feedback from MPF maintains the Ca²⁺ oscillations in fertilized ascidian eggs ensuring the eventual loss of MPF stimulating the egg-to-embryo transition. Embryonic cell cycles in the ascidian are highly stereotyped where both the rate of cell division and the orientation of cell division planes are precisely controlled. Three successive rounds of ACD generate two small posterior germ cell precursors at the 64 cell stage. The centrosome-attracting body (CAB) is a macroscopic cortical structure visible by light microscopy that causes these three rounds of ACD. Entry into mitosis activates the CAB causing the whole mitotic spindle to rotate and migrate toward the cortical CAB leading to a highly ACD whereby one small cell is formed that

A. McDougall (✉), J. Chenevert, K.W. Lee, C. Hebras, and R. Dumollard (✉)
Developmental Biology Unit UMR 7009, UMPC Univ. Paris 06 and Center National de la
Recherche (CNRS), Observatoire Océanologique, 06230 Villefranche-sur-Mer, France
e-mail: alex.mc-dougall@obs-vlfr.fr; remi.dumollard@obs-vlfr.fr

inherits the CAB and approximately 40 maternal *postplasmic/PEM* RNAs including the germ cell marker *vasa*.

8.1 Introduction to Ascidians and the Urochordates/Tunicates

Ascidians belong to the subphylum urochordata (or Tunicates) and are now thought to be the closest extant relatives of the vertebrates (Fig. 8.1 and Delsuc et al. 2006; Singh et al. 2009). Adult ascidians are sessile marine invertebrate filter feeders, which in their adult form do not resemble chordates (Fig. 8.1a).

However, ascidians form a tadpole larva (Fig. 8.1e), which has chordate structures such as a notochord and dorsal nerve tube. Ascidians have been favorites of embryologists for more than a century (Chabry 1887). A number of excellent reviews are available for those interested in ascidian embryonic development (see review by Satoh 1994, 2003; Corbo et al. 2001; Kumano and Nishida 2007; Sardet et al. 2007; Lemaire et al. 2008; Sobral et al. 2009). Since the development

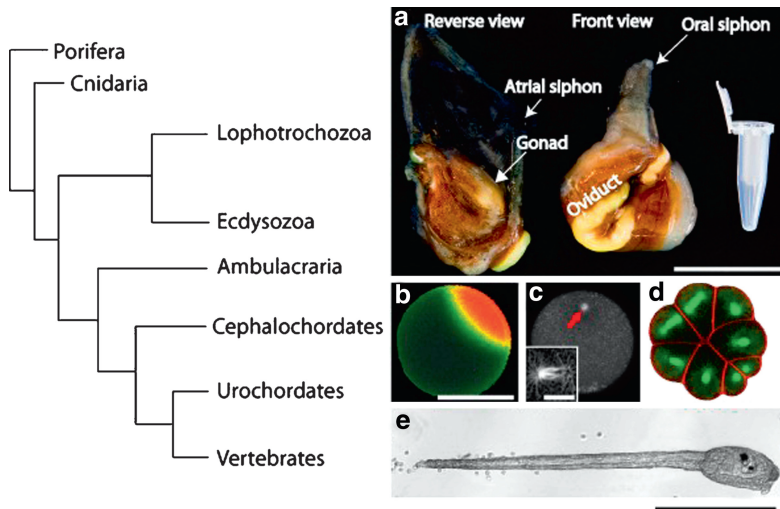


Fig. 8.1 Urochordates and the ascidian *Phallusia mammillata*. The urochordates are the closest living relative of the vertebrates [adapted from Singh et al. (2009)]. (a) The two sides of *Phallusia* following removal from its tunic (the tunic is a protective layer made of cellulose and chitin). The gonad (comprising ovary and testes) is found on the opposite side of the animal from the prominent oviduct. Mature eggs give the yellow color to the oviduct. The sperm-duct is obscured by the oviduct. Full animals contain around 100,000 eggs and about 1 mL of sperm. Scale bar = 5 cm. (b) Beginning of the sperm-triggered Ca_i^{2+} wave at fertilization (elevated Ca_i^{2+} is red). (c) Meiotic spindle (red arrow) labeled with the microtubule binding domain of MAP7 coupled to EGFP. (d) Confocal image of a 16 cell stage embryo whose microtubules are labeled green with MAP7::EGFP and the plasma membrane with the red lipophilic dye FM4-64. Scale bar = 100 μm for (b) and 5 μm for (c) inset. (e) Brightfield image of a tadpole larva approximately 15 h after fertilization. Scale bar = 100 μm

of the fate map over a century ago (Conklin 1905), many resources have been produced by the ascidian community. The initial effort for the development of online resources came from Satoh's group (Kyoto University, Japan) which culminated in the sequencing and extensive annotation of the full *Ciona intestinalis* genome by JGI (<http://genome.jgi-psf.org/ciona4/ciona4.home.html>, see Dehal et al. 2002). This revealed that the nonduplicated genome of *Ciona* (160 Mb) has approximately 16,000 genes. Other extremely useful databases are housed at Aniseed (<http://aniseed-ibdm.univ-mrs.fr/>, see Tassy et al. 2006) and the spectacular 3D virtual embryo at aniseed), Ghost (<http://ghost.zool.kyoto-u.ac.jp/>, see Satou et al. 2005) and DBTGR (<http://dbtgr.hgc.jp/>, see Sierro et al. 2006). Extensive film archives showing ascidian development (<http://biodev.obs-vlfr.fr/recherche/biomarcell/>, see Sardet et al. 2007) and 3D reconstruction of different embryonic stages are also available (<http://chordate.bpni.bio.keio.ac.jp/faba/1.1/top.html>, see Hotta et al. 2007). In addition to these online resources, germline transgenesis (see review by Sasakura 2007), electroporation (Corbo et al. 1997), knockdown using morpholinos (see Christiaen et al. 2009 for a review), blastomere manipulation (see Nishida and Sawada 2001 and other articles by Nishida), a large collection of "Gateway" reporter constructs (Roure et al. 2007), live cell imaging from mRNA encoding GFP reporter constructs in eggs (Levasseur and McDougall 2000), and early embryos (Prodon et al. 2010) are methods that are routinely applied in the ascidian model. Three species of ascidian are commonly used: *Ciona intestinalis* which is cosmopolitan and is the most popular, the Japanese ascidian *Halocynthia roretzi* which has large eggs and embryos that are ideal for micromanipulation and the European ascidian *Phallusia mammillata* which has completely transparent eggs and embryos that are ideal for imaging. *Ciona* and *Phallusia* both belong to the same order (Enterogona) while *Halocynthia* belongs to the Pleurogona. In this review we will focus on what is known about how the cell cycle is controlled from oogenesis and fertilization through early embryonic development.

8.2 Cell Cycle During Oogenesis and Fertilization

8.2.1 Oogenesis

In the ascidians, four stages of oogenesis have been described based on oocyte size, yolk content and pigmentation, cortical endoplasmic reticulum (cER) distribution, mitochondrial distribution, and DNA condensation status as well as follicular cell morphology (Fig. 8.1 and Prodon et al. 2006; Swalla et al. 1991; Jeffrey and Capco 1978). Stage I oocytes are previtellogenic and most likely represent preleptotene to pachytene stage oocytes (Fig. 8.2). Stage II is the vitellogenic stage during which oocyte volume increases dramatically.

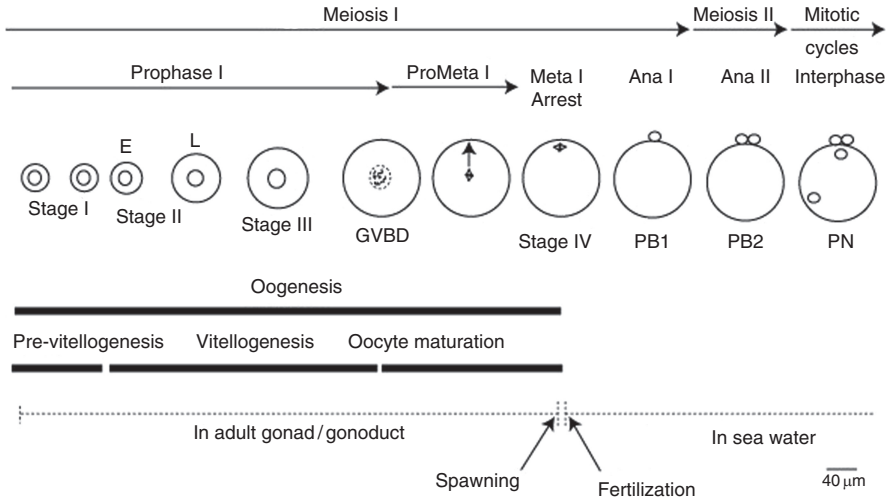


Fig. 8.2 Oogenesis and egg activation are depicted. The stages of meiosis are shown on the *top* (prophase I through to anaphase II). During prophase I the stage I oocytes are likely those undergoing the process of crossing over (preleptotene to pachytene). Once the oocytes reach stage II (diplotene) they arrest and accumulate yolk during vitellogenesis (*E*, *early* and *L*, *late*). Stage III oocytes continue to grow until they undergo GVBD. Following GVBD oocyte maturation occurs during which time the chromosomes and first meiotic spindle migrate to the oocyte cortex. Mature stage IV oocytes arrest in metaphase I and are spawned ready for fertilization. Germinal vesicle breakdown (GVBD), polar body (PB), and pronucleus (PN) fertilization. Fertilization triggers the formation of two small polar bodies (PB1 and PB2) followed by formation of the male and female pronuclei (PN stage)

8.2.2 Oocyte Maturation

Stage III oocytes are fully grown germinal vesicle (GV) oocytes at the diakinesis stage of prophase, which have a centrally located GV and a subcortical mitochondrial layer (termed the myoplasm), and are ready to mature (Conklin 1905, see Prodon et al. 2006). Stage III oocytes do not display an animal-vegetal axis and the mitochondria-rich myoplasm lines the whole of the egg subcortex (Swalla et al. 1991; Prodon et al. 2006). Stage III oocytes removed from the ovary mature spontaneously in sea water (Sawada and Schatten 1989; Sakairi and Shirai 1991; Lambert 2008; Prodon et al. 2009). Because germinal vesicle breakdown (GVBD) is very rapid in stolidobranch (a suborder of the Pleurogona) ascidians such as *Molgula* and *Boltenia* and takes up to 2.5 h in phlebobranch (a suborder of the Enterogona) ascidians such as *Ciona* and *Phallusia* it has been suggested that preMPF (maturation promoting factor) is likely present in the faster maturing group and that cyclin B synthesis is likely required in the slower maturing group (Lambert 2008). Like nemerteans (Stricker and Smythe 2001), but unlike mammals (see Mehlmann 2005 for a review), an increase in cAMP induces GVBD in the ascidian *Boltenia villosa* (Lambert 2008). Recently it has been shown that in

Ciona intestinalis oocytes a fall in cAMP levels induces GVBD as in mammalian oocytes (Silvestre et al. 2010). After GVBD, an actin-dependent mechanism is required for spindle migration toward the prospective animal pole (Prodon et al. 2006) as in mouse oocytes (Leader et al. 2002; Azoury et al. 2008; Schuh and Ellenberg 2008).

8.2.3 Sperm-Triggered Calcium Oscillations and Exit from Metaphase I Arrest in Ascidians

8.2.3.1 Metaphase I Arrest

c-Mos is a key Ser/Thr protein kinase in oocytes and is well known for its role in inducing CSF (cytostatic factor) arrest in unfertilized eggs (Sagata et al. 1989; Masui 2000 and Fig. 8.3). c-Mos is present throughout the Eumetazoa (Cnidaria: Amiel et al. 2009; Mollusc: Shibuya et al. 1992; Echiura: Gould and Stephano 1999; Echinoderms: Tachibana et al. 1997; Urochordates: Russo et al. 2009; Vertebrates: Hashimoto et al. 1994; Verlhac et al. 1996). Erp1 relays the signal from the Mos/MAPK cascade to inhibit the anaphase promoting complex/cyclo-some (APC/C^{cdc20}) thus inducing metaphase II arrest by preventing destruction of cyclin B in vertebrates (Rauh et al. 2005; Schmidt et al. 2005; Shoji et al. 2006 and Fig. 8.3). How mature ascidian eggs arrest at metaphase I is intriguing since the *Ciona intestinalis* genome does not contain Erp1 (see Fig. 8.3).

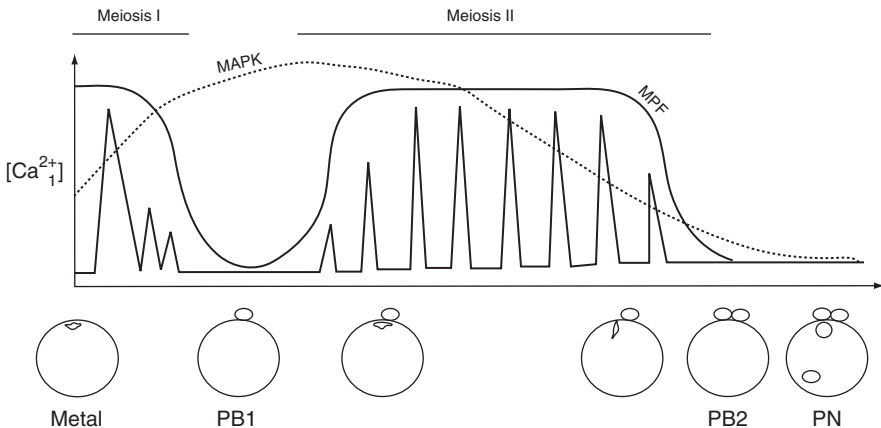


Fig. 8.3 Meta I-CSF and egg activation in ascidian eggs. Meta I-CSF is likely composed of the Mos/ERK pathway causing an inhibition of APC/C^{cdc20}, thus preventing cyclin B destruction. However, Erp1 is not present in ascidians (*dashed box*). We therefore propose that egg activation does not involve CaMKII (which operates via Erp1) and instead suggest that calcineurin (CN) is likely involved in ascidian egg activation

Sperm-triggered Ca^{2+} increases are a universal feature of egg activation (see reviews by Stricker 1999 and Whitaker 2006). In *Xenopus* eggs the sperm-triggered Ca^{2+} transient activates a kinase (calmodulin-dependent kinase II or CaMKII, Lorca et al. 1993) and a phosphatase (calcineurin or CN, Mochida and Hunt 2007; Nishiyama et al. 2007). CN and CaMKII are both required to fully activate the APC/C^{cdc20} and trigger exit from metaphase II arrest (Fig. 8.3). CaMKII phosphorylates Erp1 opening up a cryptic phosphorylation site for XPlk1 causing the proteolytic destruction of Erp1 via the β -Trcp pathway (Schmidt et al. 2005). However, it is not known whether CN or CaMKII are involved during egg activation in ascidians or other invertebrates. Because ascidians do not contain Erp1 we speculate that CaMKII is not involved in egg activation in the ascidians. On the contrary, since CN directly stimulates the APC/C we would surmise that CN is involved during egg activation in the ascidian (Fig. 8.3) and perhaps other invertebrate eggs that are stimulated to exit metaphase I by a Ca^{2+} signal (see Fig. 8.1b).

Egg activation requires more than APC/C^{cdc20} activity since the Mos/MAPK signaling cascade also has to be turned off. During egg activation in the mouse the loss of MAPK activity is required to exit meiosis and form pronuclei (Moos et al. 1995). c-Mos contains N-terminal Pro and Ser residues forming part of an N-terminal degron that controls the switch between c-Mos stability during metaphase and destruction during interphase (Sheng et al. 2002). MPF induces c-Mos stability by direct phosphorylation of Ser-3 in the N-terminal degron in *Xenopus* oocytes (Castro et al. 2001). Loss of MPF activity therefore favors c-Mos instability and leads to the proteolytic destruction of c-Mos. Since it is ERK1/2 that mediates the effect of the Mos/MAPK pathway, ERK1/2 activity has also to be switched off once c-Mos is degraded and this requires dephosphorylation of the conserved Thr or Tyr in the TEY motif of ERK1/2. In *Xenopus* oocytes protein phosphatase 2A (PP2A) causes Thr dephosphorylation of the TEY motif of ERK1/2, which is sufficient to inactivate ERK1/2 in intact oocytes (Sohaskey and Ferrell 1999). Our unpublished observations suggest that the inactivation of MPF is not a sufficient stimulus for inducing the loss of MAPK activity in ascidians caused by ERK1. However, a role of PP2A appears to be conserved between *Xenopus* and the ascidian since we find that ERK dephosphorylation depends on PP2A following egg activation (Levasseur et al. in preparation).

8.2.3.2 Sperm-Triggered Ca^{2+} Oscillations Trigger Egg Activation

Ca^{2+} oscillations are often triggered by sperm as is the case in many invertebrate species and in mammalian eggs (see reviews by Sardet et al. 1998; Dumollard et al. 2002). These Ca^{2+} oscillations are associated with the meiotic cell cycle (see review by Sardet et al. 1998; Stricker 1999). This correlation between the meiotic cell cycle and the presence of Ca^{2+} oscillations is not fortuitous since sperm-triggered Ca^{2+} oscillations are maintained when exit from the meiotic cell cycle is blocked in mouse (Jones et al. 1995) and ascidian eggs (Levasseur and McDougall 2000). In ascidians, sperm entry triggers two series of periodic Ca^{2+} waves that occur during

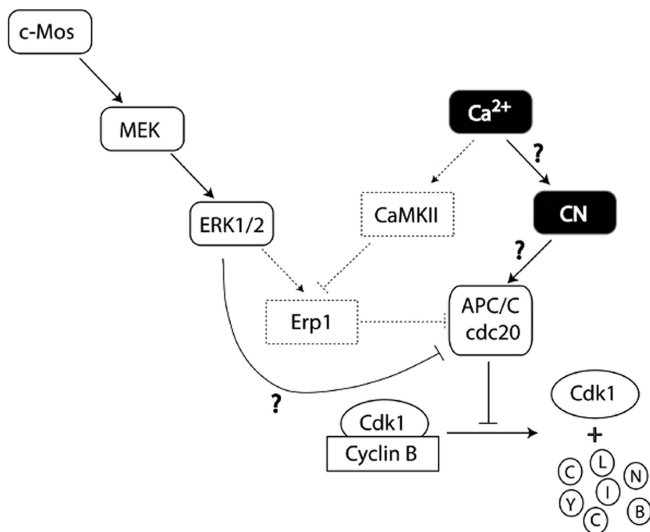


Fig. 8.4 Sperm-triggered Ca²⁺ oscillations, MPF activity, and MAPK activity are shown following fertilization. Meiosis I and II and the morphology of the egg/zygote are shown. The calcium oscillations correlate with meiosis I and II and the phases of MPF activity. Extrusion of the first and second polar bodies is indicated (PB1 and PB2) followed by formation of the pronuclei (PN)

meiosis I and II (Fig. 8.3 and Speksnijder et al. 1989a, b; McDougall and Levasseur 1998 and reviewed in McDougall 2001). These Ca²⁺ oscillations are required for meiotic exit (McDougall and Sardet 1995) and lead to the loss of MPF activity (Russo et al. 1996; McDougall and Levasseur 1998; Levasseur 1998) and an increase in the rate of cyclin B destruction (Levasseur and McDougall 2000). The two series of meiotic Ca²⁺ waves in ascidian eggs are triggered by two different localized Ca²⁺ wave pacemakers (McDougall and Sardet 1995; Dumollard and Sardet 2001). The pacemaker driving meiosis I Ca²⁺ waves is located in a cortical ER-rich domain surrounding the sperm aster and the pacemaker driving meiosis II lies in a cortical ER domain in a vegetal constriction called the contraction pole (Speksnijder et al. 1993; McDougall and Sardet 1995; Dumollard and Sardet 2001). How MPF regulates the two Ca²⁺ wave pacemakers associated with each phase of meiosis is not yet known (Levasseur et al. 2007). Both phases of Ca²⁺ waves occur when the MPF activity is elevated during meiosis I and II and pause for about 5 min when the MPF activity is low (McDougall and Levasseur 1998 and Fig. 8.4). The periodic Ca²⁺ waves finally stop just before extrusion of the second polar body when both the MPF and MAPK activities are low. Remarkably, by maintaining the MPF activity of the egg elevated the sperm-triggered Ca²⁺ waves keep pulsing across the fertilized egg indefinitely (Levasseur and McDougall 2000 and review by McDougall 2001). Because the egg can respond to the Ca²⁺-releasing second messenger inositol 1,4,5-trisphosphate (InsP₃) during the pause between the two pacemakers (Fig. 8.4), we suggested that MPF regulates InsP₃ production rather

than the sensitivity of InsP₃ receptors (McDougall and Levasseur 1998). InsP₃ receptors do eventually become desensitized upon entry into first interphase once both MAPK and MPF activities have decreased (Levasseur and McDougall 2003). The positive–negative feedback loop between MPF and the sperm-triggered Ca²⁺ waves therefore ensures that the egg-to-embryo transition occurs.

8.3 Embryonic Cell Cycles in the Ascidian

8.3.1 *The Duration and Number of Cell Cycles Are Precisely Controlled*

Not much is known about how the cell cycle is controlled during embryogenesis in the ascidians. However, the embryo displays a highly stereotyped pattern of cell division where both the rate of cell division and the orientation of cell division planes are precisely controlled. Both the rapid rate of cell division and the precise orientation of the cleavage plane are necessary for the embryo to develop rapidly with minimal proliferation. Indeed, ascidians form a tadpole larva composed of less than 2,700 cells that hatches about 12 h after fertilization (Satoh 2003). Gastrulation starts at the 110 cell stage by the invagination of 10 endodermal cells (see review by Swalla 1993 and book by Satoh 1994, page 57). At this stage, four lineages are determined and almost all cells are already fate-restricted (see Kumano and Nishida 2007 for a review).

8.3.1.1 The Rate of Cell Division

After meiotic completion, the mitotic cell cycles of the cleaving ascidian embryo show several features in common with early mitotic cell cycles found in *Xenopus* or zebrafish embryos. Like these and other embryos including mammals, the first cell cycle is longer than the subsequent ones (see Fig. 8.5 and Kubiak et al. 2008 for a review).

From the 2 cell stage to the 16 cell stage (cell cycles 2–4), the embryo displays synchronous and rapid divisions but with a slight decrease in cell cycle speed (Fig. 8.5). The progressive slowing of the cell cycle is due to an increase in interphase length (from 8 min at the 2 cell to 35 min at the 16 cell stage) whereas the duration of mitosis stays constant lasting approximately 15 min (Dumollard et al. unpublished observations). At the fifth cell cycle (16 cell stage), asynchronous cell cycles are observed when the eight animal cells divide after the eight vegetal cells giving rise to a brief 24 cell stage. This is due to the fact that mitosis begins later in the eight cells (Chenevert et al. unpublished observations). Such asynchrony follows the onset of zygotic transcription, which for the earliest genes begins at the eight cell stage (Shimauchi et al. 1997; Miya and Nishida 2003). Zygotic transcription of

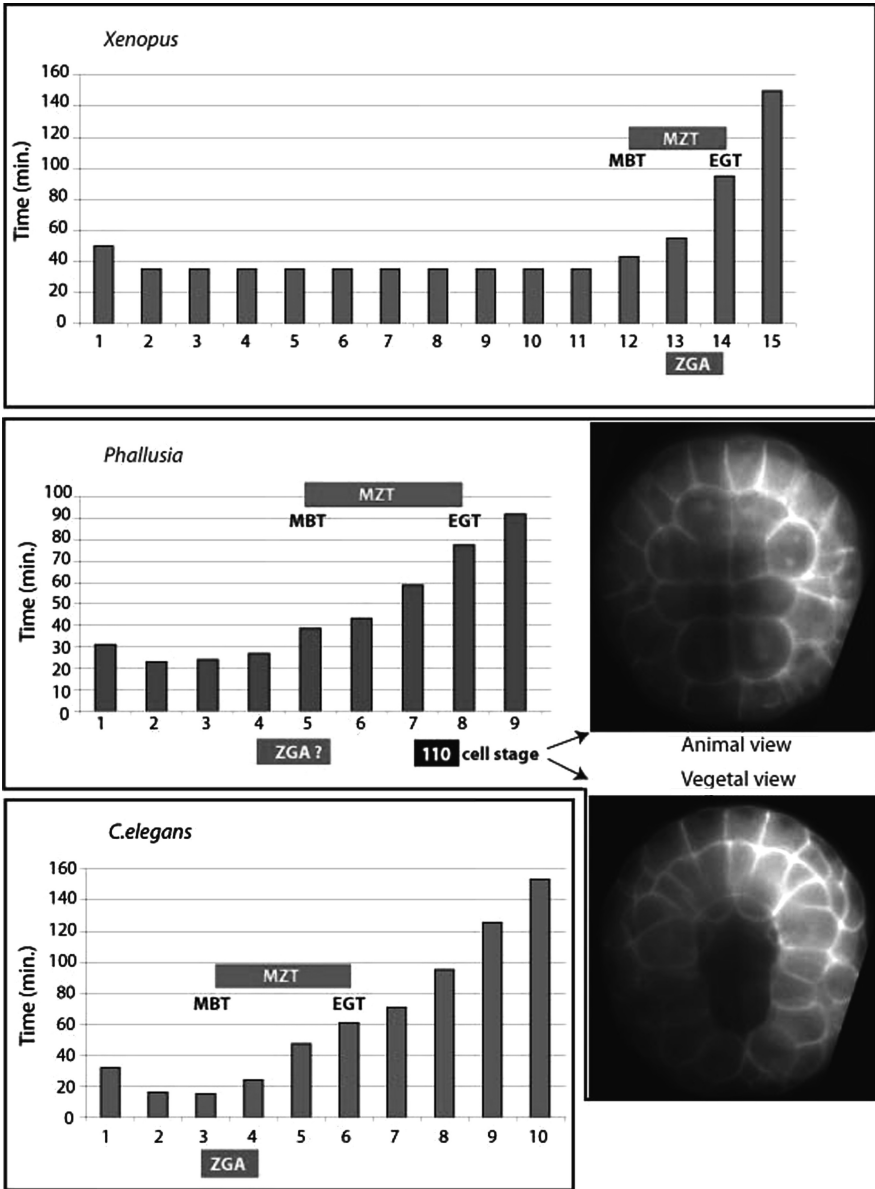


Fig. 8.5 Cell cycle duration in *Xenopus*, *Phallusia*, and *C. elegans* embryos. Midblastula transition (MBT), early gastrula transition (EGT), midzygotic transition (MZT), and zygotic genome activation (ZGA) are indicated. The x-axis shows the cell cycle number such that 1 is the 1 cell stage, 2 the 2 cell, and 3 the 4 cell stage, etc. The plasma membrane marker PH::EGFP shows the individual cells at the 110 cell stage in *Phallusia* at the onset of gastrulation. The vegetal view shows the formation of the blastopore

transcription factors increases progressively from the 16 cell stage onwards (Imai et al. 2004). There is not therefore an abrupt mid blastula transition (MBT) in the ascidian embryo as has been described in *Xenopus* (Newport and Kirschner 1982a, b), zebrafish (Kane and Kimmel 1993) and *Drosophila* cellularization (Foe and Alberts 1983). *C. elegans* embryos might also display an abrupt MBT (Bao et al. 2008 and Fig. 8.5). Nonetheless, in the ascidian as in *Xenopus*, *C. elegans* and *Drosophila*, gastrulation begins two or three cell cycles after zygotic genes are first expressed and cell cycle time begins to lengthen (Fig. 8.5).

Asynchrony is further amplified in the vegetal half of the embryo where the cells that will form the primary muscle, notochord, and endoderm precursors are located. Ascidian embryos from different orders (*Phallusia* and *Halocynthia*) display the same distinctive 110 cell stage embryo indicating that the mechanisms regulating both the rate and the orientation of cell divisions are well-conserved in solitary ascidians (Fig. 8.5). This asynchrony in the rate of cell division is maintained after gastrulation and further amplified when some cells stop dividing at the neurula stage such as the muscle and notochord lineages whilst others such as the endoderm or epidermis continue to divide during early tailbud stage. The net result of this is that a tadpole larva composed of ~2,600 cells is produced containing precisely 36 muscle cells, 40 notochord cells, 330 central nervous system cells, about 500 endoderm cells and a large number of mesenchyme and epidermal cells (see review by Satoh 2003). The ascidian embryo will be a powerful model system to decipher how cell cycle progression is modulated in each of these lineages.

8.3.1.2 The Orientation of the Cell Division Plane and the Centrosome-Attracting Body or CAB

All cleavage planes of the early ascidian embryo are precisely oriented in a defined manner. This program is essential for the segregation of developmental determinants and the morphology of the embryo. Aside from the centrosome-attracting body (CAB)-regulated divisions (see below), very little is known about the mechanisms that control positioning of the ascidian mitotic spindles during development.

The CAB is a macroscopic structure visible by light microscopy that mediates these three rounds of asymmetric cell division (ACD) (Hibino et al. 1998). In the cell in which it resides the CAB causes an unequal cleavage and is inherited by the smaller of the two daughter cells generating two small posterior cells at the 64 cell stage that are germ cell precursors (Nishikata et al. 1999). Around 40 maternal *postplasmic/PEM* (posterior end mark) RNAs including *macho-1* and *pem-1* (Yamada 2006; Prodon et al. 2007; Paix et al. 2009) as well as the germ cell marker *vasa* (Fujimura and Takamura 2000; Paix et al. 2009) are enriched in the CAB. Postplasmic/PEM RNAs were first identified as a group of maternal RNAs that localize to the posterior end mark hence the term PEMs. These *postplasmic/PEM* RNA domains are formed during oogenesis. The *postplasmic/PEM* RNAs are localized to the CAB during the 8 cell stage and tethered there until the 110 cell

stage. During the next cell cycle some *postplasmic/PEM* RNAs are segregated into a somatic cell (termed B8.11) while others including the germ cell marker *vasa* are segregated into the germ cell precursor cell (B8.12). How the CAB controls the plane of cytokinesis so that one of the two daughter cells inherits these *postplasmic/PEM* RNAs is not entirely known. However, it is known that the CAB accumulates a submembranous layer of cortical polarity proteins PAR3, PAR6, and aPKC (Patalano et al. 2006) that are known to be involved in spindle orientation in *C. elegans* one cell embryos, *Drosophila* neuroblasts, and mammalian epithelial cells (see review by Siller and Doe 2009). Classic micromanipulation experiments demonstrated that removal of the CAB abolished ACD and that transplantation of the CAB caused unequal cleavage at an ectopic site (Nishida 1994, 1996; Nishikata et al. 1999). Finally, PEM1 protein is enriched in the CAB and is required for unequal cleavage (Negishi et al. 2007).

A dual mechanism organizes the mitotic spindle during ACD in the ascidian *Phallusia mammillata* (Fig. 8.6 and Prodon et al. 2010). Previously it had been reported that a microtubule bundle dragged the nucleus toward the CAB causing the mitotic spindle to form at an asymmetric site near the CAB (Nishikata et al. 1999). However, in *Phallusia* embryos we recently found that one pole of the mitotic spindle is attracted toward the CAB after NEB from prometaphase through metaphase (Fig. 8.6 and Prodon et al. 2010). The movement of the spindle pole toward the CAB is strongly correlated with the onset of prometaphase, but it is unknown how the cell cycle affects spindle pole migration toward the CAB. The capacity of

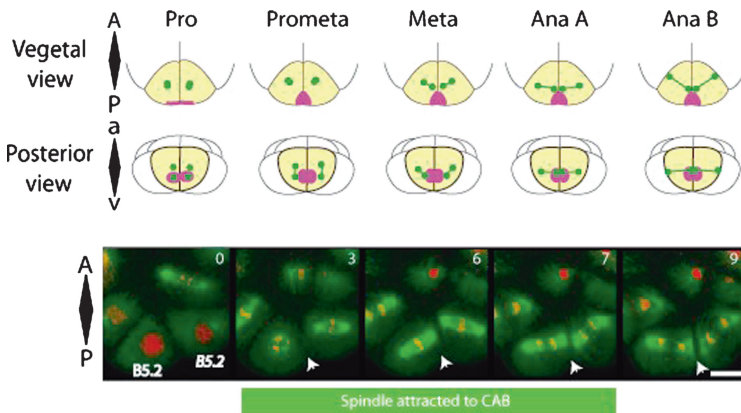


Fig. 8.6 Asymmetric cell division. *Top*: model showing vegetal and posterior views of a 16 cell stage embryo with the B5.2 cells highlighted yellow. The movement of the spindle poles (green dots) toward the CAB (violet) from prometaphase through metaphase is shown. Anterior (A), posterior (P), animal (a) and vegetal (v) are indicated. *Below*: confocal images displaying spindle alignment during mitotic progression in a pair of B5.2 cells at the 16 cell stage (vegetal view). Chromosomes are red (histone H2B::mRfp1), microtubules green (MAP7::EGFP) and the time is indicated in minutes. Arrowheads indicate CAB position. Scale bar = 20 μ m

the CAB to attract the spindle pole could be an indirect consequence of MPF's affect on microtubule reorganization that occurs during prometaphase. Alternatively, the CAB may itself be rendered competent: it is known that the CAB undergoes a cell cycle-dependent shape change and becomes compact during prometaphase when it attracts the spindle pole (Patalano et al. 2006). Interestingly, once the CAB has attracted one spindle pole the opposite pole then migrates toward the site of previous cell division and we suggest the midbody remnant may be involved (Prodon et al. 2010), as has been proposed in two cell *C. elegans* embryos (Waddle et al. 1994). Recently it has been demonstrated that both *macho-1* and β -catenin are also involved during ACD in the *Halocynthia roretzi* (Kumano et al. 2010). Knockdown of both β -catenin and *macho-1* disrupts the microtubule bundle that is proposed to drag the nucleus toward the CAB before nuclear envelope breakdown (NEB) (Kumano et al. 2010). It will also be interesting to determine how β -catenin and *Macho-1* affect the movement of the mitotic spindle toward the CAB after NEB in *Phallusia* embryos.

While cell division in CAB-containing cells is the most dramatic and extensively-studied ACD during early embryonic development in ascidians, many other cells of the early embryo also undergo ACD. Quantitative measurements of 3D models reveal other unequal cleavages (Tassy et al. 2006). Also, during the acquisition of tissue identities many binary cell fate choices are made yielding two daughter cells that have different fates such as the division that produces one notochord cell and one neural precursor cell at the 44 cell stage (Picco et al. 2007).

8.4 Conclusions and Perspectives

Valuable insight into how sperm-triggered calcium oscillations in the mammalian egg are regulated in a cell cycle-dependent manner came largely from work performed in the ascidian. It will be interesting to probe further the nature of Meta I-CSF and in particular determine how the Mos/MAPK pathway inhibits the APC/C in the absence of Erp1. Are there specific metaphase I APC coactivators such as *cortex* in *Drosophila* or Slp1 in *S. pombe*? On a related note, it will be equally interesting to determine how the fertilization Ca^{2+} signal is transduced in the absence of Erp1. Is calcineurin involved and not CaMKII? In the embryo it will be intriguing to decipher how the asynchrony in cell cycle duration arises during early embryonic development. Is cell cycle asynchrony and lengthening in ascidian embryos due to the increase in nucleocytoplasmic ratio as in *Xenopus* or is it due to unequal inheritance of maternal factors as in *C. elegans*? Finally, since it is the spindle rather than the nucleocentrosomal complex that is attracted toward the CAB, it will be possible to determine whether asymmetric spindle positioning is caused by activation of the APC/C as in one cell *C. elegans* embryos or by cell cycle regulated kinases such as Aurora A in *Drosophila* neuroblasts.

Acknowledgments We would like to thank the ANR (08-BLAN-0136-02), ARC, and ATIP for financial support. We would also like to thank Patrick Chang and Mark Levasseur for helpful comments.

References

- Amiel A, Leclère L, Robert L, Chevalier S, Houliston E (2009) Conserved functions for Mos in eumetazoan oocyte maturation revealed by studies in a cnidarian. *Curr Biol* 19:305–311
- Azoury J, Lee KW, Georget V, Rassinié P, Leader B, Verlhac MH (2008) Spindle positioning in mouse oocytes relies on a dynamic meshwork of actin filaments. *Curr Biol* 18:1514–1519
- Bao Z, Zhao Z, Boyle TJ, Murray JI, Waterson RH (2008) Control of cell cycle timing during *C. elegans* embryogenesis. *Dev Biol* 318:65–72
- Castro A, Peter M, Magnaghi-Jaulin L, Vigneron S, Galas S, Lorca T, Labbe JC (2001) Cyclin B/cdc2 induces c-Mos stability by direct phosphorylation in *Xenopus* oocytes. *Mol Biol Cell* 12:2660–2671
- Chabry LM (1887) Contribution à l'embryologie normale tératologique des ascidies simples. *J Anat Physiol Norm Pathol* 23:167–321
- Christiaen L, Wagner E, Shi W, Levine M (2009) Microinjection of morpholino oligos and RNAs in sea squirt (*Ciona*) embryos. *Cold Spring Harb Protoc* 12
- Conklin EG (1905) The organization and cell lineage of the ascidian egg. *J Acad Sci Philadelphia* 13:1–119
- Corbo JC, Levine M, Zeller RW (1997) Characterization of a notochord-specific enhancer from the Brachyury promoter region of the ascidian, *Ciona*. *Development* 124:589–602
- Corbo JC, Di Gregorio A, Levine M (2001) The ascidian as a model organism in developmental and evolutionary biology. *Cell* 106:535–538
- Dehal P, Satou Y, Campbell RK et al (2002) The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. *Science* 298:2157–2167
- Delsuc F, Brinkmann H, Chourrout D, Philippe H (2006) Tunicates and not cephalochordate are the closest living relatives of vertebrates. *Nature* 439:965–968
- Dumollard R, Sardet C (2001) Three different calcium-wave pacemakers in ascidian eggs. *J Cell Sci* 114:2471–2481
- Dumollard R, Carroll J, Dupont G, Sardet C (2002) Calcium wave pacemakers in eggs. *J Cell Sci* 115:3557–3564
- Foe VE, Alberts BM (1983) Studies on nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. *J Cell Sci* 61:31–70
- Fujimura M, Takamura K (2000) Characterization of an ascidian DEAD-box gene, Ci-DEAD1: specific expression in the germ cells and its mRNA localization in the posterior most blastomeres in early embryos. *Dev Genes Evol* 210:64–72
- Gould MC, Stephano JL (1999) MAP kinase, meiosis, and sperm centrosome suppression in *Urechis caupo*. *Dev Biol* 216:348–358
- Hashimoto N, Watanabe N, Furuta Y, Tamemoto H, Sagata N, Yokoyama M, Okazaki K, Nagayoshi M, Takeda N, Ikawa Y et al (1994) Parthenogenetic activation of oocytes in c-mos-deficient mice. *Nature* 370:68–71
- Hibino T, Nishikata T, Nishida H (1998) Centrosome-attracting body: a novel structure closely related to unequal cleavages in the ascidian embryo. *Dev Growth Differ* 40:85–95
- Hotta K, Mitsuhara K, Takahashi H, Inaba K, Oka K, Gojobori T, Ikeo K (2007) A web-based interactive developmental table for the ascidian *Ciona intestinalis*, including 3D real-image embryo reconstructions: I. From fertilized egg to hatching larva. *Dev Dyn* 236:1790–1805

- Imai KS, Hino K, Yagi K, Satoh N, Satou Y (2004) Gene expression profiles of transcription factors and signaling molecules in the ascidian embryo: towards a comprehensive understanding of gene networks. *Development* 131:4047–4058
- Jeffrey WR, Capco DG (1978) Differential accumulation and localization of maternal poly(A)-containing RNA during early development of the ascidian *Styela*. *Dev Biol* 67:152–166
- Jones KT, Carroll J, Merriman JA, Whittingham DG, Kono T (1995) Repetitive sperm-induced Ca^{2+} transients in mouse oocytes are cell cycle dependent. *Development* 121:3259–3266
- Kane DA, Kimmel CB (1993) The zebrafish midblastula transition. *Development* 119:447–456
- Kubiak JZ, Ciemerych MA, Hupalowska A, Sikora-Polaczek M, Polanski Z (2008) On the transition from the meiotic to mitotic cell cycle during early mouse development. *Int J Dev Biol* 52:201–217
- Kumano G, Nishida H (2007) Ascidian embryonic development: an emerging model system for the study of cell fate specification in chordates. *Dev Dyn* 236:1732–1747
- Kumano G, Kawai N, Nishida H (2010) *Macho-1* regulates unequal cell divisions independently of its function as a muscle determinant. *Dev Biol* 344(1):284–292
- Lambert CC (2008) Signaling pathways in ascidian oocyte maturation: the role of cyclic AMP and follicle cells in germinal vesicle breakdown. *Dev Growth Differ* 50:181–188
- Leader B, Lim H, Carabatsos MJ, Harrington A, Ecsedy J, Pellman D, Maas R, Leder P (2002) *Formin-2*, polyploidy, hypofertility and positioning of the meiotic spindle in mouse oocytes. *Nat Cell Biol* 4:921–928
- Lemaire P, Smith WC, Nishida H (2008) Ascidiaceans and the plasticity of the chordate developmental program. *Curr Biol* 18:R620–R631
- Levasseur M, McDougall A (2000) Sperm-induced calcium oscillations at fertilisation in ascidians are controlled by cyclin B1-dependent kinase activity. *Development* 127:631–641
- Levasseur M, McDougall A (2003) IP_3 responsiveness is regulated in a meiotic cell cycle dependent manner: implications for fertilisation induced calcium signaling. *Cell Cycle* 2:610–614
- Levasseur M, Carroll M, Jones KT, McDougall A (2007) A novel mechanism controls the Ca^{2+} oscillations triggered by activation of ascidian eggs and has an absolute requirement for CDK1 activity. *J Cell Sci* 120:1763–1771
- Lorca T, Cruzalegui FH, Fesquet D, Cavadore J-C, Mery J, Means A, Doree M (1993) Calmodulin-dependent protein kinase II mediates inactivation of MPF and CSF upon fertilisation of *Xenopus* eggs. *Nature* 366:270–273
- Masui Y (2000) The elusive cytotstatic factor in the animal egg. *Nat Rev Mol Cell Biol* 1:228–232
- McDougall A (2001) Sperm-triggered calcium oscillations at fertilization. In: *The biology of ascidians*. Springer, pp 36–46
- McDougall A, Levasseur M (1998) Sperm triggered calcium oscillations during meiosis in ascidian oocytes first pause, restart then stop: correlations with cell cycle kinase activity. *Development* 125:4451–4459
- McDougall A, Sardet C (1995) Function and characteristics of repetitive calcium waves associated with meiosis. *Curr Biol* 5:318–328
- Mehlmann LM (2005) Stops and starts in mammalian oocytes: recent advances in understanding the regulation of meiotic arrest and oocyte maturation. *Reproduction* 130:791–799
- Miya T, Nishida H (2003) Expression pattern and transcriptional control of *SoxB1* in embryos of the ascidian *Halocynthia roretzi*. *Zoolog Sci* 20:59–67
- Mochida S, Hunt T (2007) Calcineurin is required to release *Xenopus* egg extracts from meiotic M phase. *Nature* 449:336–340
- Moos J, Visconti PE, Moore GD, Schultz RM, Kopf GS (1995) Potential role of mitogen-activated protein kinase in pronuclear envelope assembly and disassembly following fertilization of mouse eggs. *Biol Reprod* 53:692–699
- Negishi T, Takada T, Kawai N, Nishida H (2007) Localized PEM mRNA and protein are involved in cleavage-plane orientation and unequal cell divisions in ascidians. *Curr Biol* 17:1014–1025

- Newport J, Kirschner M (1982a) A major developmental transition in early *Xenopus* embryos. I. Characterization and timing of cellular changes at the midblastula stage. *Cell* 30:675–686
- Newport J, Kirschner M (1982b) A major developmental transition in early *Xenopus* embryos. II. Control of the onset of transcription. *Cell* 30:687–696
- Nishida H (1994) Localization of determinants for formation of the anterior-posterior axis in eggs of the ascidian *Halocynthia roretzi*. *Development* 120:3093–3104
- Nishida H (1996) Vegetal egg cytoplasm promotes gastrulation and is responsible for specification of vegetal blastomeres in embryos of the ascidian *Halocynthia roretzi*. *Development* 122:1271–1279
- Nishida H, Sawada K (2001) *macho-1* encodes a localized mRNA in ascidian eggs that specifies muscle fate during embryogenesis. *Nature* 409:724–729
- Nishikata T, Hibino T, Nishida H (1999) The centrosome-attracting body, microtubule system, and posterior egg cytoplasm are involved in positioning of cleavage planes in the ascidian embryo. *Dev Biol* 209:72–85
- Nishiyama T, Yoshizaki N, Kishimoto T, Ohsumi K (2007) Transient activation of calcineurin is essential to initiate embryonic development in *Xenopus laevis*. *Nature* 449:341–345
- Paix A, Yamada L, Dru P, Lecordier H, Pruliere G, Chenevert J, Satoh N, Sardet C (2009) Cortical anchorages and cell type segregations of maternal postplasmic/PEM RNAs in ascidians. *Dev Biol* 336:96–111
- Patalano S, Prulière G, Prodon F, Paix A, Dru P, Sardet C, Chenevert J (2006) The aPKC-PAR-6-PAR-3 cell polarity complex localizes to the centrosome-attracting body, a macroscopic cortical structure responsible for asymmetric divisions in the early ascidian embryo. *J Cell Sci* 119:1592–1603
- Picco V, Hudson C, Yasuo H (2007) Ephrin-Eph signalling drives the asymmetric division of notochord/neural precursors in *Ciona* embryos. *Development* 134:1491–1497
- Prodon F, Chenevert J, Sardet C (2006) Establishment of animal-vegetal polarity during maturation in ascidian oocytes. *Dev Biol* 290:297–311
- Prodon F, Yamada L, Shirae-Kurabayashi M, Nakamura Y, Sasakura Y (2007) Postplasmic/PEM RNAs: a class of localized maternal mRNAs with multiple roles in cell polarity and development in ascidian embryos. *Dev Dyn* 236:1698–1715
- Prodon F, Hanawa K, Nishida H (2009) Actin microfilaments guide the polarized transport of nuclear pore complexes and the cytoplasmic dispersal of *Vasa* mRNA during GVBD in the ascidian *Halocynthia roretzi*. *Dev Biol* 330:377–388
- Prodon F, Chenevert J, Hébras C, Dumollard R, Faure E, Gonzalez-Garcia F, Nishida H, Sardet C, McDougall A (2010) Dual mechanism controls asymmetric spindle position in ascidian germ cell precursors. *Development* 137:211–221
- Rauh NR, Schmidt A, Bormann J, Nigg EA, Mayer TU (2005) Calcium triggers exit from meiosis II by targeting the APC/C inhibitor XErp1 for degradation. *Nature* 437:1048–1052
- Roure A, Rothbacher U, Robin F, Kalmar E, Ferone G, Lamy C, Missero C, Mueller F, Lemaire P (2007) A multicassette Gateway vector set for high throughput and comparative analyses in *ciona* and vertebrate embryos. *PLoS One* 2(9):e916
- Russo GL, Kyoizuka K, Antonazzo L, Tosti E, Dale B (1996) Maturation promoting factor in ascidian oocytes is regulated by different intracellular signals at meiosis I and II. *Development* 122:1995–2003
- Russo GL, Bilotto S, Ciarcia G, Tosti E (2009) Phylogenetic conservation of cytostatic factor related genes in the ascidian *Ciona intestinalis*. *Gene* 429:104–111
- Sagata N, Watanabe N, Vande Woude GF, Ikawa Y (1989) The *c-mos* proto-oncogene product is a cytostatic factor responsible for meiotic arrest in vertebrate eggs. *Nature* 342:512–518
- Sakairi K, Shirai H (1991) Possible MIS production by follicle cells in spontaneous oocyte maturation of the ascidian *Halocynthia roretzi*. *Dev Growth Differ* 33:155–162
- Sardet C, Roegiers F, Dumollard R, Rouiere C, McDougall A (1998) Ca^{2+} waves and oscillations in eggs. *Biophys Chem* 72:131–140

- Sardet C, Paix A, Prodon P, Chenevert J (2007) From oocyte to 16-cell stage: cytoplasmic and cortical reorganizations that pattern the ascidian embryo. *Dev Dyn* 236:1716–1731
- Sasakura Y (2007) Germline transgenesis and insertional mutagenesis in the ascidian *Ciona intestinalis*. *Dev Dyn* 236:1758–1767
- Satoh N (1994) *Developmental biology of ascidians*. Cambridge University Press, New York
- Satoh N (2003) The ascidian tadpole larva: comparative molecular development and genomics. *Nat Rev Genet* 4:285–295
- Satou Y, Kawashima T, Shoguchi E, Nakayama A, Satoh N (2005) An integrated database of the ascidian, *Ciona intestinalis*: towards functional genomics. *Zool J Linn Soc* 8:837–843
- Sawada T-O, Schatten G (1988) Microtubules in ascidian eggs during meiosis, fertilization and mitosis. *Cell Motil Cytoskeleton* 9:219–230
- Sawada T-O, Schatten G (1989) Effects of cytoskeletal inhibitors on ooplasmic segregation and microtubule organization during fertilization and early development in the ascidian *Molgula occidentalis*. *Dev Biol* 132:331–342
- Schmidt A, Duncan PI, Rauh NR, Sauer G, Fry AM, Nigg EA, Mayer TU (2005) Xenopus polo-like kinase Plx1 regulates XErp1, a novel inhibitor of APC/C activity. *Genes Dev* 19:502–513
- Schuh M, Ellenberg J (2008) A new model for asymmetric spindle positioning in mouse oocytes. *Curr Biol* 18:1986–1992
- Sheng J, Kumagi A, Dunphy WG, Varshavsky A (2002) Dissection of c-MOS degron. *EMBO J* 21:6061–6071
- Shibuya EK, Boulton TG, Cobb MH, Ruderman JV (1992) Activation of p42 MAP kinase and the release of oocytes from cell cycle arrest. *EMBO J* 11:3963–3975
- Shimauchi Y, Yasuo H, Satoh N (1997) Autonomy of ascidian fork head/HNF-3 gene expression. *Mech Dev* 69:143–154
- Shoji S, Yoshida N, Amanai M, Ohgishi M, Fukui T, Fujimoto S, Nakano Y, Kajikawa E, Perry AC (2006) Mammalian Emi2 mediates cytostatic arrest and transduces the signal for meiotic exit via Cdc20. *EMBO J* 25:834–845
- Sierro N, Kusakabe T, Park KJ, Yamashita R, Kinoshita K, Nakai K (2006) DBTGR: a database of tunicate promoters and their regulatory elements. *Nucleic Acids Res* 34:D552–D555
- Siller KH, Doe CQ (2009) Spindle orientation during asymmetric cell division. *Nat Cell Biol* 11:365–374
- Silvestre F, Gallo A, Cuomo A, Covino T, Tosti E (2010) Role of cyclic AMP in the maturation of *Ciona intestinalis* oocytes. *Zygote* Sep 2:1–7 [Epub ahead of print]
- Singh TR, Tsagkogeorga G, Delsuc F, Blanquart S, Shenkar N, Loya Y, Douzery EJ, Huchon D (2009) Tunicate mitogenomics and phylogenetics: peculiarities of the *Herdmania momus* mitochondrial genome and support for the new chordate phylogeny. *BMC Genomics* 10:534
- Sobral D, Tassy O, Lemaire P (2009) Highly divergent gene expression programs can lead to similar chordate larval body plans. *Curr Biol* 19:2014–2019
- Sohaskey ML, Ferrell JE Jr (1999) Distinct, constitutively active MAPK phosphatases function in xenopus oocytes: implications for p42 MAPK regulation in vivo. *Mol Biol Cell* 10:3729–3743
- Speksnijder JE, Corson W, Sardet C, Jaffe LF (1989a) Free calcium pulses follow fertilisation in the ascidian egg. *Dev Biol* 135:182–190
- Speksnijder JE, Jaffe LF, Sardet C (1989b) Polarity of sperm entry in the ascidian egg. *Dev Biol* 133:180–184
- Speksnijder JE, Terasaki M, Hage WJ, Jaffe LF, Sardet C (1993) Polarity and reorganisation of the endoplasmic reticulum during fertilisation and ooplasmic segregation in the ascidian egg. *J Cell Biol* 120:1337–1346
- Stricker SA (1999) Comparative biology of calcium signaling during fertilisation and egg activation in animals. *Dev Biol* 211:157–176
- Stricker SA, Smythe TL (2001) 5-HT causes an increase in cAMP that stimulates, rather than inhibits, oocyte maturation in marine nemertean worms. *Development* 128:1415–1427
- Swalla BJ (1993) Mechanisms of gastrulation and tail formation in ascidians. *Microsc Res Tech* 26:274–284

- Swalla BJ, Badgett MR, Jeffery WR (1991) Identification of a cytoskeletal protein localized in the myoplasm of ascidian eggs: localization is modified during anural development. *Development* 111:425–436
- Tachibana K, Machida T, Nomura Y, Kishimoto T (1997) MAP kinase links the fertilization signal transduction pathway to the G1/S-phase transition in starfish eggs. *EMBO J* 16:4333–4339
- Tassy O, Daian F, Hudson C, Bertrand V, Lemaire P (2006) A quantitative approach to the study of cell shapes and interactions during early chordate embryogenesis. *Curr Biol* 16:345–358
- Verlhac MH, Kubiak JZ, Weber M, Geraud G, Colledge WH, Evans MJ, Maro B (1996) Mos is required for MAP kinase activation and is involved in microtubule organization during meiotic maturation in the mouse. *Development* 122:815–822
- Waddle JA, Cooper JA, Waterston RH (1994) Transient localized accumulation of actin in *Caenorhabditis elegans* blastomeres with oriented asymmetric divisions. *Development* 120:2317–2328
- Whitaker M (2006) Calcium at fertilization and in early development. *Physiol Rev* 86:25–88
- Yamada L (2006) Embryonic expression profiles and conserved localization mechanisms of pem/postplasmic mRNAs of two species of ascidian, *Ciona intestinalis* and *Ciona savignyi*. *Dev Biol* 296:524–536