

# Maternal determinants and mRNAs in the cortex of ascidian oocytes, zygotes and embryos

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## Abstract

The peripheral region of ascidian oocytes and zygotes contains five determinants for morphogenesis and differentiation of the embryo. The determinant for the 24 primary muscle cells of the tadpole, *macho1*, is one of several cortical mRNAs localized in a gradient along the animal–vegetal axis in the oocyte. After fertilization these mRNAs, together with cortical endoplasmic reticulum (cER) and a subcortical mitochondria-rich domain (myoplasm), relocate in two major reorganization phases forming the posterior plasm (postplasm) of the zygote. At the 8-cell stage cortical mRNAs concentrate in a macroscopic cortical structure called the centrosome-attracting body (CAB), forming a characteristic posterior end mark (PEM) in the two posterior vegetal blastomeres. We propose to call the numerous mRNAs showing this particular cortical localization in the posterior region of the embryo *postplasmic/PEM* RNAs and suggest a nomenclature. We do not know how *postplasmic/PEM* RNAs reach their polarized distribution in the oocyte cortex but at least *PEM1* and *macho1* (and probably others) bind to the network of cER retained in isolated cortical fragments. We propose that after fertilization, these *postplasmic/PEM* mRNAs move in the zygote cortex together with the cER network (cER/mRNA domain) via microfilament- and microtubule-driven translocations. The cER/mRNA domain is localized posteriorly at the time of first cleavage and distributed equally between the first two blastomeres. After the third cleavage, the cER/mRNA domain and dense particles compact to form the CAB in posterior vegetal blastomeres of the 8-cell stage. We discuss the identity of *postplasmic/PEM* RNAs, how they localize, anchor, relocate and may be translated. We also examine their roles in unequal cleavage and as a source of posterior morphogenetic and differentiation factors.

## Introduction

### A little history: ascidians and the notion of cortical maternal determinants

The first embryo manipulations (destruction of blastomeres) were performed on *Ascidia aspersa* embryos by Laurent Chabry, a French biologist, in 1887 (Chabry,

1887). Chabry showed that individual remaining blastomeres invariably developed into partial larvae. The American embryologist Edwin Conklin agreed that ascidian embryos resulted from a type of development he qualified as ‘mosaic’ and suggested that factors, or ‘organ forming substances’, were located in ‘organ forming regions’ (in this review we call these regions domains) (Conklin, 1905b). In his monumental publications on *Styela (Cynthia) partita* development, Conklin proposed that the essential factors were located at the periphery of the egg in several domains: ectoplasm, endoplasm etc. Conklin thought

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**Key words:** development, egg, endoplasmic reticulum, polarity, RNA localization.

**Abbreviations used:** CAB, centrosome-attracting body; cER, cortical endoplasmic reticulum; EST, expressed sequence tag; GEF, guanine nucleotide-exchange factor; PEM, posterior end mark; PVC, posterior-vegetal cytoplasm; UTR, untranslated region.

that the essential factors for muscle development were located in a yellow-pigmented domain called the 'crescent of mesodermal substance' (later called 'myoplasm') which segregated into tail muscle cells (Conklin, 1905a). Conklin also noted a small clear peripheral domain beneath the myoplasm which we will describe as the cortical endoplasmic reticulum (cER)/mRNA-rich domain in this review (remarkable reproductions of Conklin's original drawings are on the web site <http://zygote.swarthmore.edu/Conklin/Conklin.html>). In the 1950s and 1960s Italian embryologists, led by the cleric Guiseppe Reverberi, showed that early blastomeres made of recognizable cytoplasmic and cortical domains, had particular cell fates and differentiated into embryonic tissues even when raised in isolation (cell-autonomous fate specification; Reverberi, 1956). In the 1980s and 1990s embryologists in the U.S.A. and Japan performed the first *in situ* localizations of specific mRNAs, mapped maternal determinants in the periphery of the egg and zygotes, provided accurate cell lineages for early blastomeres and clarified whether they differentiated because they inherited maternal factors ('determinants') and/or needed additional induction signals from neighbouring blastomeres (reviewed in Jeffery, 1995; Nishida, 1997; Nishida et al., 1999; Satoh, 1994). The role of the cytoskeleton and cortex in re-localizing domains and maternal determinants after fertilization was also elucidated (Sardet et al., 1989; Sawada and Schatten, 1989; Swalla et al., 1991). However, the identity of the 'organ forming substances' remained mysterious until specific cortical mRNAs with developmental effects and the molecular nature of the first determinant (for muscle differentiation) were discovered (Nishida and Sawada,

2001; Yoshida et al., 1996). In recent years the publication of the *Ciona intestinalis* genome, which comprises about 15 000 genes (there are no major duplication events in contrast to vertebrates), the constitution of large collections of ESTs (expressed sequence tags) from eggs, zygotes, embryonic stages and adult tissues of different types in several solitary and colonial ascidian species, and the first reports on mutants and transgenics, has stimulated an increasing number of biologists to use the ascidian model (Imai et al., 2004; Makabe et al., 2001; Matsuoka et al., 2004; Satoh, 2003; Satoh et al., 2003).

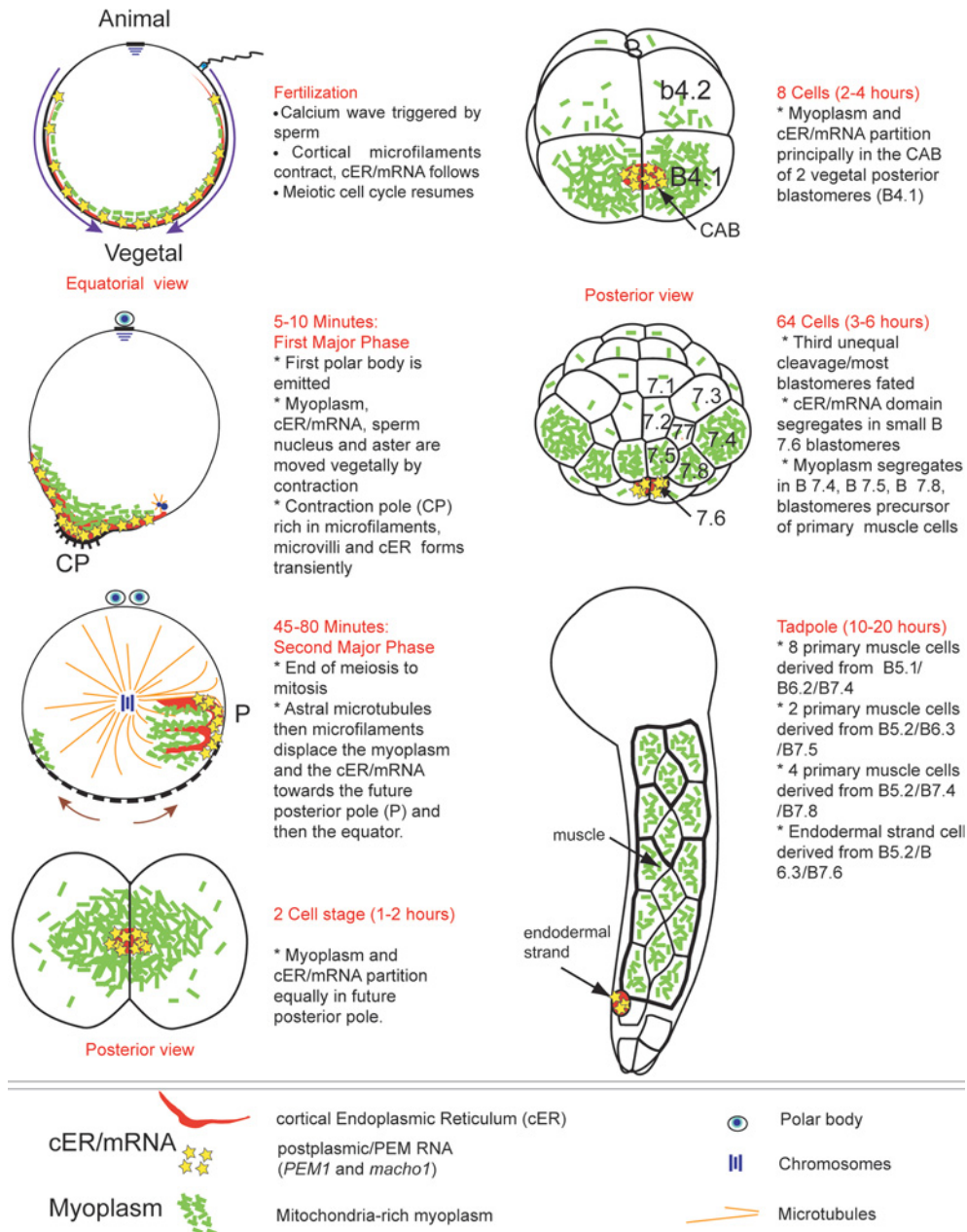
### The discovery of developmentally important cortical mRNAs in ascidians

The discovery of specific cortical mRNAs in *Ciona savignyi* called 'posterior end mark' (PEM) RNAs by Noriyuki Satoh and his colleagues took place in a favourable context. In the 1980s and 1990s it was shown that two major developmental models (the insect *Drosophila melanogaster* and amphibian *Xenopus laevis*) relied on cortically-localized mRNAs (Bicoid, Oskar, Vg1 . . .) for axis establishment and germ layer specification (see our site <http://biodev.obs-vlfr.fr/biomarcell:embryo-comparisons-poster>). During the same period Hiroki Nishida performed exquisite ablations of specific peripheral regions of the large (280 µm) eggs and zygotes of *Halocynthia roretzi* (reviewed in Nishida, 1997; Nishida, 2002b; Nishida et al., 1999). These experiments showed that determinants for three cell types (ectoderm, endoderm, primary muscle) and two morphogenetic processes (posterior unequal cleavages, gastrulation) were located within 10–20 µm of the surface, and relocalized

**Maternal determinants:** Maternal determinants are factors (mRNAs, proteins or their complexes) inherited from the oocyte which direct axis formation and/or the differentiation of tissues in the embryo. When maternal determinants are inhibited or removed, axis formation or development are abnormal. When determinants are injected or induced in ectopic blastomeres they can redirect developmental fate.

**The ascidian model:** Ascidians are sessile invertebrates (*Urochordata*, *Tunicata*, *Asciidiacea*) which develop into tadpoles with the basic body plan of chordates, but are simply made of 2600 cells and six tissue types (Satoh, 1994). Out of more than 2300 recognized species six species of solitary (*C. intestinalis* and *C. savignyi*, *Halocynthia roretzi*, *Phallusia mamillata*, *Asciodiella aspersa*) and colonial (essentially *Botryllus schlosseri*) ascidians are used for research (<http://biodev.obs-vlfr.fr/biomarcell/ascidies/species.html>). The fertilized egg goes through a series of stereotyped equal and then unequal divisions to rapidly reach the 110-cell stage (onset of gastrulation). At that stage, the developmental fates of all easily recognized blastomeres is determined by inheritance of maternal factors and/or interactions between blastomeres (Nishida, 1997; 2002a; 2002b; Satoh, 2003; Satoh et al., 2003; see Figure 1). The tadpole attaches to a substrate and metamorphoses into a juvenile in 1–3 days. These juveniles grow into reproductive adults in 1–3 months. Micromanipulations (ablations, transfers, blastomere isolations and reassociations) are easily performed on eggs and large quantities of synchronous zygotes and embryos can be raised. The first mutants and transgenics have been reported recently (reviewed in Satoh et al., 2003). In recent years *Ciona intestinalis* a cosmopolitan species has been chosen as a model for whole genome analyses and gene network analysis (Satoh et al., 2003; Imai et al., 2004). Abundant information on genes and ESTs can be found on the following sites: <http://genome.jgi-psf.org/ciona4/ciona4.home.html>, and <http://ghost.zool.kyoto-u.ac.jp/index1.html>. For those interested in ascidian development, the egg cortex and in multimedia documents we recommend to visit our site <http://biodev.obs-vlfr.fr/recherche/biomarcell/>, watch the 'clip of the month': 'Reorganizations in ascidians after fertilization' and the egg cortex page: <http://biodev.obs-vlfr.fr/recherche/biomarcell/ascidies/eggcortex.html>. A part of the cER/mRNA story is told in the form of a 5 minute multimedia BioClip 'Polarity in the egg cortex' which can be downloaded from: <http://www.bioclips.com/bioclips.html>.

**Figure 1 | Schematic representation of the localization and reorganizations of *postplasmic/PEM* RNAs (yellow stars) in eggs and zygotes of ascidians, and their segregation in posterior blastomeres of embryos and tissues of tadpoles, together with peripheral cER-rich domain (in red) and mitochondria-rich myoplasm domain (in green)**  
 The cER/mRNA domain (red region with yellow stars) is shown as a precursor of the CAB.



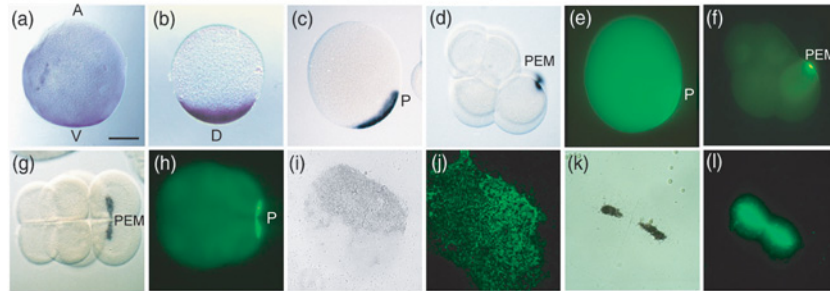
after fertilization at the same time as the peripheral domain called myoplasm relocated (a process called ‘ooplasmic segregation’ see Figure 1 and Figure 2). Specific mRNAs belonging to the *pem* family (see Definition and Table 1) were first identified from

an RNA differential screening experiment involving myoplasm-rich fragments obtained by centrifugal fragmentation of *C. savignyi* eggs (merogones) (Yoshida et al., 1996; 1997). Comparisons of mRNAs specifically localized in myoplasm-rich fragments led

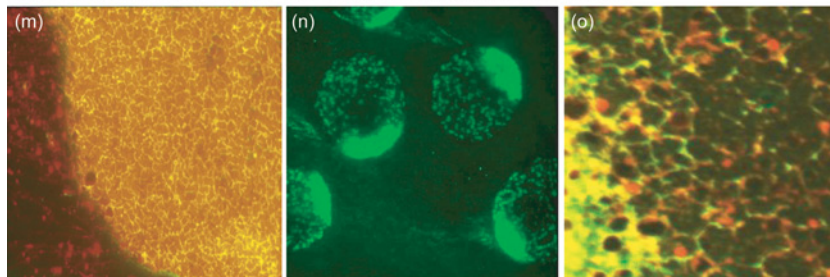
**Figure 2 | Localization of mRNAs by *in situ* hybridization (fluorescent TSA and alkaline phosphatase precipitation methods) in oocytes, zygotes and embryos and tadpoles of 3 different species (*C. intestinalis*, *H. roretzi* and *P. mammillata*)**

Colocalizations of cER and *PEM1* RNA are shown in isolated cortical fragments derived from oocytes, zygotes and 8-cell stage embryos of *Ciona* and *Halocynthia*. Figures 2d–2h 2m and 2o are reprinted with permission (The Company of Biologists) from Sardet et al., Development 2003 130: 5839–5849.

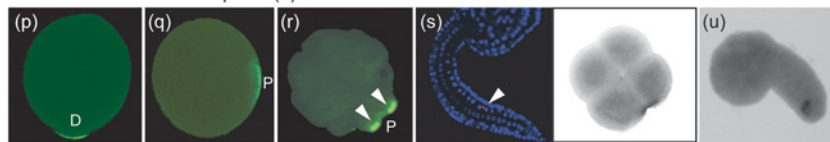
*Halocynthia roretzi* *PEM1* and *macho1* in oocytes (a), zygotes (b, c, e), 8 cell embryos (d, f, g, h) and isolated cortices from zygotes (i, j) and posterior blastomeres of 8 cell stages (k, l) (from Sardet et al., 2003). A: Animal, V: Vegetal, D: Dorsal, P: Posterior, PEM: Posterior End Mark



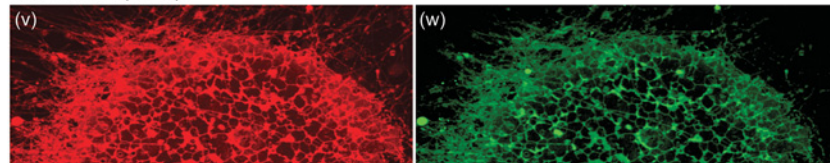
Isolated cortices from oocytes (m), zygotes (n) and edge of CAB region of posterior blastomeres of 8 cell embryo of *Halocynthia roretzi* retain *PEM1* mRNA (green) bound to the cER network (red). Merged cER and *PEM1* images in yellow (m, o) (from Sardet et al., 2003).



*Ciona intestinalis* *PEM1* in zygotes after first (p) and second phase (q) of reorganization, in embryos (r) and in endodermal cells of tadpoles (s, arrowhead). CAB where *PEM1* RNAs accumulate is indicated by arrowheads. *Phallusia mammillata* *Vasa* and *Tolloid* in embryos (t) and in endodermal cells of tadpoles (u).



Isolated cortices from oocytes of *Ciona intestinalis* retain *PEM1* mRNA (w, green) bound to the cER network (v, red).



to the discovery of the first and most abundant *pem* (*pem* or as we suggest *Cs-PEM1*; see Definition and Table 1) and its effect on anterior–posterior development. This important discovery was followed by the identification of six mRNAs with the same localiz-

ation pattern and coding for proteins, with (*Wnt5*, *MEX3*) or without similarities to other proteins known at that time (Nakamura et al., 2003; Sasakura et al., 1998; Satou, 1999; Satou and Satoh, 1997). More than 20 such cortical maternal mRNAs have

now been detected in the cosmopolitan species *C. intestinalis* and *C. savignyi* and in the Japanese ascidian *H. roretzi* (Table 1 and see below).

Using the large *Halocynthia* zygotes, Nishida and his colleagues demonstrated that when they ablated the region containing the posterior plasm (posterior-vegetal cytoplasm; abbreviated PVC) primary muscle cell differentiation did not occur. Ablation of the PVC also radialized the embryo (i.e. embryos lost the ability to undergo three unequal cleavages in the posterior region between the 8–64-cell stages: reviewed in Nishida, 1997; Nishida, 2002a; Nishida, 2002b). Fragments of the zygotes containing the PVC could be re-inserted by fusion to the anterior region of an embryo with transfer of unequal cleavage and muscle cell forming potential to the anterior, proving that determinant factors were located in the PVC. Subtractive hybridization screening comparing animal and vegetal blastomeres of the large *Halocynthia* embryos at the 8-cell stage led to the discovery of *macho1* mRNA in 2001 (Nishida and Sawada, 2001). Ability to inhibit muscle differentiation by use of anti-sense induction of muscle cell differentiation by ectopic injection of *macho1* mRNA in anterior animal blastomeres (which normally never give muscle cells) and other arguments (see Nishida, 2002a; Nishida, 2002b) showed that *macho1* mRNA was an essential component of the long awaited muscle determinant in *Halocynthia*. This was further confirmed in *C. intestinalis* and *savigny* (Satou et al., 2002). *Macho1* codes for a transcription factor of the Zic family (important for vertebrate neuronal differentiation) and has a general influence on posterior tissue differentiation. Its influence in ascidian embryos is greater than that originally described and its mode of action is debated (Kobayashi et al., 2003; Kondoh et al., 2003; Nishida, 2002a).

The mRNAs which were specifically located in the posterior plasm of *Halocynthia* zygotes were called postplasmic mRNAs due to their localization in the PVC (Sasakura et al., 2000). Since many of these RNAs were shown to have the same identity in three different ascidians species (*PEM3*, *Wnt5*, *macho1*, etc.

see Table 1) and the same characteristic peripheral distribution and displacements, we propose calling this class of cortical maternal messenger RNAs *postplasmic/PEM* RNAs, and to adopt a common nomenclature for them (see Definition below and Table 1).

We have observed that the European ascidian *Phallusia mammillata* also possesses *postplasmic/PEM* RNAs (*Tolloid-like*, *Vasa-like*, *macho1-like*; C. Sardet, P. Dru and F. Prodon, unpublished data). Therefore it seems that although *Cionas*, *Phallusia* (phlebobranch and aplousobranch ascidians/*Enterogona*) and *Halocynthia* (stolidobranch ascidian/*Pleurogona*) are separated from each other by millions of years of evolution, these ascidian species have conserved some basic molecular and cellular patterning essential for early development (see Figure 1). These different ascidian species probably rely on a conserved set of maternal *postplasmic/PEM* RNAs for development (Table 1). This is clearly a favourable situation for analysing conserved mechanisms of localization, anchoring, relocation and translation of mRNAs and their mode of action (Sardet et al., 2003; Sasakura and Makabe, 2002; Tanaka et al., 2004). This analysis is underway in the small but fast-growing ascidian researcher community.

### Characterization of *postplasmic/PEM* RNAs (Figure 2 and Table 1)

We have compiled a list of *postplasmic/PEM* RNAs which takes into account published reports, and information available in the databases for *C. intestinalis*, *C. savignyi* and *H. roretzi* (see Table 1). The estimated abundance of transcripts is obtained from EST databases since it has been shown that the relative frequency of identified EST clones can be taken as a rough measure of transcript abundance (Satou et al., 2003). Using that criterion, as well as the strength of the *in situ* hybridization signal, it is clear that *PEM1* and *PEM3* are the most abundant *postplasmic/PEM* RNAs in both *Cionas* and in *Halocynthia* eggs and in early cleavage stages. These abundant cortical maternal mRNAs remain in the smaller cells in posterior-most position in the embryo (micromeres

**A proposed common nomenclature for cortically-localized maternal mRNAs that form a posterior end mark in ascidian embryos:** The nomenclature used in this field is presently confusing. The first highly localized mRNA originally discovered in *Ciona savignyi* was named *pem* (because it was visualized as a 'posterior end mark' by *in situ* localizations of 8-cell stage embryos). Similarly localized mRNAs were called *pem-2*, *-3*, *-4*, etc. and *PEM1*, *PEM3* etc. in *Halocynthia*. In *Halocynthia* these RNAs were designated as 'postplasmic' RNAs because they were localized in the posterior plasm. We designate these cortically-localized maternal mRNAs as *postplasmic/PEM* RNAs. We have compiled the original names given to these maternal RNAs in Table 1 and propose a common nomenclature (*Ci-PEM*, *Hr-PEM*, etc.) presented in Table 1 (see nomenclature in the legend of the table).

**Table 1 | Nomenclature and compilation of *Postplasmic/PEM* RNAs in eggs and embryos of three species of ascidians**

The common nomenclature we propose is based on the species name abbreviation (*Ci*, *Cs*, *Hr*, *Pm*, etc.) followed by a hyphen, as used in recent gene compilations (Imai et al., 2004) and as suggested at a recent gathering (International Urochordate Meeting 2003, Carry Le Rouet, Marseille, France). This is followed by capital letters (e.g. for Posterior End Mark) using italics (genes transcripts) and a number (e.g. *Ci-PEM1*, *Cs-PEM1*, *Hr-PEM1*, etc.) unless (or until) the gene has a name related to its function (e.g. *Ci-macho1*, *Hr-macho1*) or corresponds to an already identified gene (e.g. *Ci-Wnt5*, *Hr-Wnt5*, *Ci-Tolloid*).

Name		Identification				CAC repeats in 3'UTR		
		NCBI ACC Number*	cDNA projects cluster ID†‡	Gene ID in <i>Ciona intestinalis</i> genome	Abundance (frequency in egg EST database)	REPFIND P-Score§	CAC repeats/3'UTR length   (CAC repeats in cluster/ cluster length)	CAC rich cluster % of 3'UTR
<i>PEM1</i> (the original and most abundant <i>postplasmic/PEM</i> , it plays a role in antero–dorsal patterning)								
<i>Ci-pem</i>	<i>Ci-PEM1</i>	<a href="#">AK113383</a>	CLSTR1544	ci0100145638	~ 1/1000	$3.43 \times 10^{-3}$	18/728 (7/87)	0.12
<i>pem</i>	<i>Cs-PEM1</i>	<a href="#">BAA11926.1</a>				$2.74 \times 10^{-4}$	20/1230 (10/164)	0.13
<i>HrPEM</i>	<i>Hr-PEM1</i>	<a href="#">AB045129</a>	C0011		~ 1/1000	$4.10 \times 10^{-8}$	24/920 (17/235)	0.26
<i>PEM2</i> (codes for a GEF)								
	<i>Ci-PEM2</i>	<a href="#">AK113181</a>	CLSTR04638					
		<a href="#">AK114933</a>	05668, 10565	ci0100132337	~ 1/1500	$2.83 \times 10^{-2}$	12/469 (8/177)	0.38
<i>pem-2</i>	<i>Cs-PEM2</i>	<a href="#">AB001770</a>				$2.33 \times 10^{-4}$	16/694 (10/130)	0.19
<i>PEM3</i> [codes for an RNA-binding protein ( <i>MEX3/TINO</i> -like)]								
	<i>Ci-PEM3</i>	<a href="#">AK114726</a>	CLSTR0865	ci0100139193	~ 1/1000	$2.92 \times 10^{-2}$	5/627 (3/35)	0.06
<i>pem-3</i>	<i>Cs-PEM3</i>	<a href="#">AB001769</a>				$2.14 \times 10^{-3}$	7/545 (3/11)	0.02
<i>HrPEM-3</i>	<i>Hr-PEM3</i>	<a href="#">AB091123</a>			~ 1/1000	$2.98 \times 10^{-3}$	26/2465 (8/185)	0.08
<i>PEM4</i>								
	<i>Ci-PEM4</i>	<a href="#">AK113491</a>	CLSTR6630	ci0100134675	~ 1/30 000	$1.95 \times 10^{-2}$	5/317 (2/5)	0.02
<i>pem-4</i>	<i>Cs-PEM4</i>	<a href="#">AB001771</a>				$1.68 \times 10^{-1}$	14/597 (4/67)	0.11
<i>PEM5</i>								
	<i>Ci-PEM5</i>	<a href="#">AK115396</a>	CLSTR03294	ci0100135082	~ 1/2500	$3.38 \times 10^{-1}$	6/338 (4/126)	0.37
<i>pem-5</i>	<i>Cs-PEM5</i>	<a href="#">AB001772</a>				$1.00 \times 10^{-1}$	5/323 (3/51)	0.16
<i>PEM6</i>								
	<i>Ci-PEM6</i>	<a href="#">AK113695</a>	CLSTR0813	ci0100152704	~ 1/2500	$6.44 \times 10^{-2}$	12/860 (4/76)	0.09
<i>pem-6</i>	<i>Cs-PEM6</i>	<a href="#">AB001773</a>				$1.31 \times 10^{-3}$	14/835 (3/10)	0.01
<i>macho1</i> (codes for a <i>Zic</i> -like transcription factor, muscle determinant and posterior development patterning factor)								
<i>Ci-macho1</i>	<i>Ci-macho1</i>	<a href="#">AB077368</a>	CLSTR09919	ci0100150779	~ 1/10 000	$3.60 \times 10^{-3}$	11/552 (4/24)	0.04
<i>Cs-macho1</i>	<i>Cs-macho1</i>	<a href="#">AB057740</a>				$1.77 \times 10^{-3}$	11/536 (6/74)	0.14
<i>macho-1</i>	<i>Hr-macho1</i>	<a href="#">AB045124</a>			~ 1/3000	$2.13 \times 10^{-4}$	6/489 (6/99)	0.2

POPK1 (codes for a kinase with regions homologous to SAD-1)								
<i>Ci</i> POPK-1	<i>Ci</i> -POPK1	<a href="#">AK116009</a>	CLSTR14100	ci0100133458	~ 1/30000	$7.88 \times 10^{-4}$	11/496 (10/163)	0.33
<i>Hr</i> POPK-1	<i>Hr</i> -POPK1	<a href="#">AB014885</a>	C00232		~ 1/3000	$6.35 \times 10^{-3}$	27/1670 (22/884)	0.53
Wnt5 (codes for secreted signalling glycoprotein)								
<i>Ci</i> -Wnt5	<i>Ci</i> -Wnt5	<a href="#">AF176668</a>	CLSTR07785					
			CLSTR33732	ci0100135747	~ 1/30000	$2.30 \times 10^{-4}$	14/710 (11/185)	0.26
<i>Hr</i> Wnt-5	<i>Hr</i> -Wnt5	<a href="#">AB072595</a>						
		<a href="#">AB006608</a>	C00264		~ 1/3000	$2.24 \times 10^{-5}$	8/579 (3/23)	0.04
PEN1 (codes for Zinc Finger g1-related protein)								
	<i>Ci</i> -PEN1		CLSTR1933	ci0100132333	~ 1/15000	$9.32 \times 10^{-2}$	4/247 (3/35)	0.14
<i>Hr</i> PEN-1	<i>Hr</i> -PEN1	<a href="#">AB091124</a>			~ 1/15000	$3.64 \times 10^{-3}$	11/605 (9/224)	0.37
ZF1 (codes for and RNA-binding protein with C3H type zinc finger motifs found in <i>Musashi</i> or <i>PIE-1</i> )								
	<i>Ci</i> -ZF1	<a href="#">AK113919</a>	CLSTR06030	ci0100130897	~ 1/15000	$1.97 \times 10^{-4}$	9/751 (5/41)	0.05
<i>Hr</i> ZF-1	<i>Hr</i> -ZF1	<a href="#">AB029332</a>	C01649		~ 1/15000	$1.69 \times 10^{-2}$	18/1769 (8/194)	0.11
DII-B (codes for transcription factor with LIM Zn binding and Hox domains)								
<i>Ci</i> -DII-B	<i>Ci</i> -DII-B		CLSTR11516	ci0100149957	< 1/30000	$9.76 \times 10^{-3}$	9/220 (3/13)	0.06
Tolloid [codes for secreted metalloprotease (BMP family)]								
<i>Ci</i> -Tolloid	<i>Ci</i> Tolloid		CLSTR00215	ci0100137986	~ 1/30000	$2.51 \times 10^{-4}$	17/654 (9/105)	0.16
Eph1 (codes for tyrosine kinase receptor for Ephryns, cell surface-associated ligands)								
<i>Eph1</i>	<i>Ci</i> -Eph1	<a href="#">AK116571</a>	CLSTR11732	ci0100141382	~ 1/30000	$2.80 \times 10^{-2}$	13/1236 (2/6)	0
<i>Cs</i> -EPH	<i>Cs</i> -Eph1	<a href="#">AB057735</a>				$1.20 \times 10^{-4}$	12/458 (5/25)	0.05
GLUT (codes for glucose transporter protein)								
	<i>Ci</i> -GLUT	<a href="#">AK116680</a>	CLSTR1635	ci0100144859	~ 1/15000	$1.89 \times 10^{-4}$	16/954 (11/195)	0.2
<i>Hr</i> GLUT	<i>Hr</i> -GLUT	<a href="#">AB091122</a>	C00248		~ 1/3000	$3.21 \times 10^{-3}$	19/1183 (6/87)	0.07

\**Ciona intestinalis*, *Ciona savignyi*, *Halocynthia roretzii* (<http://www.ncbi.nlm.nih.gov/entrez>).

†*Ciona intestinalis* (<http://ghost.zool.kyoto-u.ac.jp/indexr1.html>).

‡*Halocynthia roretzii* (<http://www.genome.jp/magest/>).

§The calculations used for deriving P-scores determine the probability of finding a cluster of a given motif (CAC) within a distance of x nucleotides (Betley et al., 2002).

||This notation [e.g. 18/728 (7/87)] means that for *Ci*-PEM, 18 CAC repeats are found in a 728 nt long 3'UTR (7 CAC are found in a cluster of 87 nt).

B7.6; see Figure 1). The B7.6 micromeres which retain maternal *postplasmic/PEM* RNAs stop dividing and give rise to 2 to 4 so-called endodermal strand cells at the tip of the tadpole tail (reviewed in Nishida, 1997; Davidson and Levine, 2003). On the basis of their polarized position, division pattern, translational silencing and accumulation of *Ci-DEAD1* (*Vasa-like*) mRNA (a *postplasmic/PEM* mRNA, see below) these endodermal strand cells have been proposed to be precursors of the germ line (Takamura et al., 2002; Tomioka et al., 2002) and have even been called 'pole cells' recently (by analogy with pole cells which become germ cells in *Drosophila*) (Davidson and Levine, 2003).

It is worthwhile noting that some of the maternal *postplasmic/PEM* mRNAs (particularly those genes with developmentally significant orthologues like *Wnt5*) have zygotic counterparts which are expressed in different tissues of the tadpole (Imai et al., 2004; Sasakura and Makabe, 2001; Nakamura et al., 2003). We must also stress that two types of *postplasmic* mRNAs have been distinguished in *Halocynthia*: *Type I postplasmic/PEM* RNAs (those we mainly consider in this review) are already localized in the cortex of the oocyte at the time of fertilization whereas *Type II postplasmic/PEM* RNAs are evenly distributed in the mature oocyte's cytoplasm and concentrate in the posterior/CAB (centrosome-attracting body) region after cleavage (Sasakura and Makabe, 2002; Sasakura et al., 2000). This points to the existence of several classes of *postplasmic/PEM* RNAs which we discuss in later sections.

### **Type I postplasmic/PEM RNAs (see Figure 2)**

We will review the collection (approx. 24) of *postplasmic/PEM* RNAs (using our suggested nomenclature: see Definition and Table 1) as it stands today. It is likely that the major *postplasmic/PEM* RNAs have been identified but that others which are much less abundant will emerge from the systematic *in situ* hybridization screenings (see for example Imai et al., 2004) or scanning of RNA UTR (untranslated region) sequences presently underway.

#### **PEM1**

The first described [Figures 2a–2d] and 2p–2r] and most abundant cortical mRNA (original *pem* discovered in *C. savignyi*) has no clear homologies to

those of known proteins. The ectopic injection of synthetic *pem* causes a shift of the larvae anterior neural and epidermal tissues towards the posterior (Yoshida et al., 1996). In contrast with *PEM1*, several other *Type I postplasmic/PEM* mRNAs have interesting orthologues.

#### **PEM2**

Codes for a protein with characteristic DH-PH catalytic domains conserved in mammalian proteins which belong to the large family of GEF (guanine nucleotide-exchange factor) proteins (nucleotide exchange factors including VAV, SOS, Tiam-1 and the Cdc42 human orthologue hPEM-2) (Reid et al., 1999; Satou and Satoh, 1997). These GEFs activate G proteins (e.g. Rac, Rho, Cdc42) essential for cell motility and polarization via their modulation of the actin microfilament cytoskeleton. It is interesting to note that ascidians possess all the different types of GEF present in vertebrates but have a single representative of each instead of multiple variants found in the different vertebrate species (Philips et al., 2003).

#### **PEM3 (TINO/MEX-3)**

This is an abundant *postplasmic/PEM* RNA. *PEM3* codes for an ARN-binding protein (ARE). ARE proteins bind to AU-rich elements in 3'UTRs of mRNAs via 2 single-standed RNA-binding motifs (KH domains). The regions of *PEM3* which contain the KH domains show high homology (around 70%) to two RNA-binding proteins in mammals (TINO) and in the nematode *Caenorhabditis elegans* (MEX-3 for Muscle in EXcess) (Donnini et al., 2004; Nakamura et al., 2003; Satou, 1999). The mammalian *PEM3* orthologue TINO binds to the 3'UTR of *BCL2* and controls expression of this important mitochondrial membrane protein implicated in apoptosis and myogenic differentiation. The worm orthologue, MEX-3 is located in germ granules (P granules) of the nematode embryo and is segregated to posterior germ cells (Draper et al., 1996). It controls the expression and localization of the cell fate determinant *PAL-1* via binding of its 3'UTR. *Ciona's Ci-PEM3*, *Cs-PEM3* (Satou, 1999) are also expressed zygotically and in *Halocynthia*, *Hr-PEM3* is expressed in the mesenchyme (Nakamura et al., 2003).

#### **Macho1**

*Macho1* (Figures 2g and 2h) has attracted most attention as a muscle determinant and encodes a zinc

finger protein of the *Zic* family and is probably a transcription factor (Nishida and Sawada, 2001; Satou et al., 2002). The middle portion of the *Macho1* protein is quite well conserved in *C. intestinalis*, *C. savignyi* and *H. roretzi* and most resembles *Zic5*. In both *C. intestinalis* and *C. savignyi*, *macho1* is in addition expressed zygotically from the tailbud stage, in cells of the central nervous system.

### Wnt5

Belongs to the *Wnt* family of genes which code for secreted signalling glycoproteins necessary for cell morphogenesis during gastrulation, tail formation or axon guidance in zebrafish, *Xenopus* and *Drosophila* embryos respectively. *Hr-Wnt5* is a typical *Type I postplasmic/PEM* RNA and remains in the descendants of B7.6 micromeres, marking the endodermal strand cells at the tip of the tail. The function of *Wnt5* in ascidians is not yet known but it has been recently hypothesized that a localized source of *Wnt5* in 'pole cells' may influence heart development by playing a role in the specification of the cardiac mesoderm (Davidson and Levine, 2003; Sasakura et al., 1998a). There is also a zygotic expression of *Wnt5* in both *Ciona* and *Halocynthia*. In *Halocynthia* the zygotic expression starts at the 32-cell stage in B6.1 and B6.2 posterior blastomeres and then occurs transiently in ectoderm, mesoderm and endoderm at gastrulation. Expression persists in notochord cells of the tailbud where *Wnt5* has been shown to play a role in morphogenetic movements of notochord cells (Sasakura and Makabe, 2001). In *C. intestinalis* zygotic expression begins at the 32-cell stage in B6.1 and B6.2 blastomeres and is seen in A7.4, A7.8, B7.3, B7.7 and B7.8 (see Figure 1) and in muscle and posterior epidermis at later stages (Imai et al., 2004)

### POPK1

*POPK1* (posterior pole kinase-1) was originally discovered in *Halocynthia* and recently identified in *Ciona* (Sasakura et al., 2003); it codes for a putative serine/threonine kinase. The *POPK1* RNA localizes in *Halocynthia* as a typical *postplasmic/PEM* RNA in the mature oocyte cortex, the CAB in B4.1 blastomeres, B7.6 micromeres and in endodermal strand cells derived from them. It is also expressed zygotically in neural tissues at the tailbud stage. *Hr-POPK1* codes for a protein with strong similarity to *C. elegans* *SAD-1*, a kinase which regulates presynaptic ves-

icle clustering and axon termination. Together with *Drosophila* and human homologues, the proteins encoded by *Hr-POPK1* and *Ce-SAD-1* share a conserved N-terminal kinase domain (more than 82% identity). This kinase domain also shows similarity to that of *PAR-1*, an essential polarity protein of the *MARK* family of proteins localized in the posterior cortex of the *Drosophila* oocyte and the *C. elegans* zygote (Cheeks et al., 2004; Riechmann et al., 2002). *POPK1* could play an important role in development and in the integrity of the CAB (H. Nishida, personal communication).

### GLUT

This mRNA codes for a protein with homology to a glucose transporter. It is also expressed zygotically in the mesenchyme of *Halocynthia* embryo (Nakamura et al., 2003).

### ZF-1

Encodes an RNA-binding protein with C3H-type zinc finger motifs found in *Musashi* or *PIE-1* (an important transcriptional repressor in the posterior blastomeres precursor of germ cells in *C. elegans*). It has been suggested it could bind to other *postplasmic/PEM* RNAs and play a role in their stability (Sasakura and Makabe, 2002).

### PEN-1 and -2

*PEN-1* (for posterior end) is a  $\beta$ -1-related protein also expressed zygotically in the ectoderm of *Halocynthia* embryo (Nakamura et al., 2003). It apparently has orthologues in *C. savignyi* and *C. intestinalis* (L. Yamada, personal communication).

### Tolloid, Eph1, Dll-B (all included in Table 1), Nemo-like, Fli/ERG4, FoxJ2, CGNF, Prd-B, prep, Raf1, scalloped/TEF1, Zinc Finger(C2H2)-33

These RNAs have been recently reported to localize like *postplasmic/PEM* RNAs based on an *in situ* screen for localized transcription factors and signalling macromolecules but their analysis has not yet been completed (Imai et al., 2004).

### PEM4-PEM9

Not a great deal is known about these *postplasmic/PEM* RNAs. Some (*PEM4*, *PEM5*, *PEM6*) were described

succinctly based on the PEM they made in *in situ* localizations carried out in 8-cell stage blastomeres of *C. savignyi* (Satou and Satoh, 1997). Others (*PEM7*, *PEM8*, *PEM9*) have been proposed as possible new members of the *postplasmic/PEM* family from recent compilations and localizations of *C. intestinalis* genes (Nishikata et al., 2001). The status of some of these mRNAs such as *PEM4*, *PEM5* in *C. intestinalis* (once proposed as a homologue of the *Spires* gene in *Drosophila*) is in doubt and others such as *PEM7* could be a *Type II postplasmic/PEM* RNAs (L. Yamada, personal communication and see also: Nishikata et al., 2001). There may also be species differences that need to be investigated.

On the basis of analysis of the frequency of CAC repeats in the 3' UTR region using REPFIND, a computer program which helps to identify repeat motifs in RNAs (Betley et al., 2002), it appears that *PEM4* and *PEM5* have a higher 'P' score than all other *postplasmic/PEM* RNAs (see Table 1). This indicates that they do not share the high CAC repeat frequencies in 3' or 5' UTRs which characterize mRNAs that localize in the periphery of chordate oocytes and somatic cells (Betley et al., 2002; Claussen et al., 2004).

### **Type II postplasmic/PEM RNAs and Vasa-like RNAs**

As mentioned previously, some mRNAs are not initially located in the cortex of the oocyte and zygote but localize progressively in the posterior cortex of embryos starting at the 2–4-cell stage. The first three *Type II postplasmic/PEM* RNAs were originally characterized in *Halocynthia* and named *HrPet 1*, *HrPet 2*, *HrPet 3* for posterior end translocation (Sasakura et al., 2000) (we propose they be renamed *Hr-PET1*, *Hr-PET2*, *Hr-PET3*; see Definition and Table 1). *Postplasmic/PEM* RNAs are distributed throughout the cytoplasm of unfertilized eggs, and after fertilization undergo some kind of vegetal then posterior translocation during ooplasmic segregation, where the cytoskeleton seems to be involved (Sasakura et al., 2000). Of particular interest in this category is *Ci-DEAD-1*, a *Vasa-like* mRNA which has been reported to follow *Type II postplasmic/PEM* RNA localization pattern in *C. intestinalis* (Fujimura and Takamura, 2000). Its accumulation in the posterior-most blastomeres at the 16–64-cell stage (B5.2/B6.3/B7.6) and localization in their endodermal strand cells' descendants has led to the hypothesis that B7.6

micromeres and their descendants (described as endodermal strand cells; see Figure 1) are the precursor of ascidian germ cells, a hypothesis that has received some experimental support (Fujimura and Takamura, 2000; Takamura et al., 2002), but still needs confirmation and, in particular, completion of cell lineage analysis. We should mention that in the European ascidian *P. mammillata*, *Vasa* (*Pm-Vasa*) is situated in the oocyte cortex and, at low resolution behaves like a *Type I postplasmic/PEM* mRNA (C. Djediat, C. Sardet, P. Dru, unpublished data). However, when observed at higher resolution *Pm-Vasa* is located further from the plasma membrane than *PEM1*. This and other observations suggest that a germ plasm-like domain may exist in the cortex of ascidian oocytes at the time of fertilization and that it is later located in the small posterior blastomeres; in fact, in *Phallusia* it is possible to discern five stratified domains in the vegetal peripheral region of the zygote at the time of meiosis completion (Roegiers et al., 1999).

Based on these observations, and others detailed below, we predict that there may be subclasses (*Type Ia*, *Ib*, etc.) of *postplasmic/PEM* mRNAs which will represent preferential association of specific mRNAs with some or all of the five surface/cortical/subcortical domains one can distinguish in the zygote at the end of meiosis completion (vegetal button stage; see Roegiers et al., 1999).

### **Where, when and how are postplasmic/PEM RNAs located in the oocyte cortex?**

*Type I postplasmic/PEM* RNAs considered in this review are present at the periphery of the mature oocyte and excluded from the animal pole region, forming a basket-like shell opened in the animal pole region (Figures 1 and 2). Unfortunately localization of mRNAs with low resolution *in situ* methods (visualization of the signal by enzymic precipitation) does not allow the determination of the exact topographical position of *postplasmic/PEM* RNAs with respect to the egg surface and peripheral domains (myoplasm, cER-rich domain, etc.). High resolution imaging using fluorescent *in situ* localization and confocal microscopy shows that at least *Hr-PEM1*, *Hr-macho1* (Sardet et al., 2003), *Ci-PEM1*, and *Ci-PEM3* form a reticulated network situated within 1–2  $\mu\text{m}$  of the egg surface (F. Prodon, C. Sardet and P. Dru, unpublished data). This spatial distribution suggested to us that *PEM-1* and some other

*postplasmic/PEM* RNAs might be associated with the network of cER that we knew to be distributed along an animal–vegetal gradient immediately beneath the plasma membrane of the egg of the European species *P. mammillata* (Sardet et al., 1992). High resolution observations of cortical fragments isolated from *H. roretzi* and more recently *C. intestinalis* (Figures 2m–2o, 2v and 2w; F. Prodon, C. Sardet and P. Dru, unpublished data) using special fluorescent *in situ* localization methods which preserved the cER network show that *Hr-PEM1*, *Hr-Macho1* (Sardet et al., 2003) and *Ci-PEM1* are bound to the cER network which is itself attached to cytoplasmic side of the plasma membrane (Sardet et al., 1992). Preliminary experiments also suggest that ribosomes which are abundant on the cER may be involved in the attachment of some *postplasmic/PEM* RNAs (Sardet et al., 2002) and (F. Prodon, C. Sardet and P. Dru, unpublished data). It is tempting to speculate that ER-associated RNP (ribonucleoprotein) complexes such as those containing RNA-binding proteins (Staufen/FMRP etc.) as described in plants, mammalian neurons and *Xenopus* oocytes (Belanger et al., 2003; Ohashi et al., 2002; Allison et al., 2004) are involved in this association.

It remains to be seen how many of the identified *postplasmic/PEM* RNAs are associated with the cER and how they become associated and/or polarized with the cER network. In line with initial indications (Yoshida et al., 1997) our recent observations on *C. intestinalis* (F. Prodon, C. Sardet, unpublished data) support the fact that peripheral localization and polarization of RNAs takes place during maturation. However, much remains to be done to characterize and understand this cortical localization process using strategies and techniques which are presently used to elucidate mechanisms of mRNA localization in *Xenopus* and *Drosophila* oocytes (reviewed in Kloc et al., 2002; Chang et al., 2004).

Little work has been done on the question of how these maternal mRNAs reach the ascidian egg cortex. Soon after their discovery it was noted that all *Cs-PEM* RNAs shared common UUAUUU motifs in their 3'UTR which might be involved in their localization (Satou and Satoh, 1997). The only experimental study on this question indicates that in *Halocynthia* the necessary information to localize injected mRNAs (*Hr-Wnt5* and *Hr-POPK1*) in the cortex is indeed located in their 3'UTR (Sasakura and Makabe, 2002).

For both *Hr-Wnt5* and *Hr-POPK-1* mRNAs, UG-rich repeat elements (UGREs) seem necessary (but not sufficient) to bring about localization in the egg cortex and subsequent accumulation in the posterior region like that of the endogenous RNAs. It has also been shown that the injected 3'UTR of *Hr-macho1* is able to localize in the vegetal pole region of *Xenopus* oocytes like the endogenous maternal mRNAs (*Vg1*, *VegT*, *Xpat*, *Xcat-2*, *Xdazl*) which naturally locate in the vegetal cortex of *Xenopus* (Betley et al., 2002). High frequency of CAC-rich repeats in 3'UTR (and also 5'UTR of XNIF; see Claussen et al., 2004), which apparently confer cortical localization to mRNAs in *Xenopus*, is also present in the 3'UTRs of chicken  $\beta$ -actin and zebrafish *Vasa* which localize at the periphery of fibroblasts and early embryos respectively. The analysis of 3'UTR regions of all these cortically-localized mRNAs has led to the concept that a ubiquitously conserved signal for cortical mRNA localization may be shared by chordates and that this may be encoded by regions with a high density of CAC repeats (XCACX or CAC-rich clusters). We have systematically screened the 3'UTR regions of 15 *postplasmic/PEM* mRNAs from *Cionas intestinalis* and *C. savignyi* and *H. roretzi* with REPFIND (Betley et al., 2002) to search for CAC-rich motifs and analyse their frequency and clustering pattern (Table 1). It is clear that most *postplasmic/PEM* RNAs (except for *PEM4* and *PEM5*) have much higher frequency of CAC repeats (P score values below 0.01) than would be expected from random occurrence (P score values approx. 0.1). However, the number, clustering pattern and the position of the CAC repeat clusters within the 3'UTRs of orthologues in the 3 ascidian species examined is rather variable (results not shown). It is noticeable that the UG-rich region necessary for localization in *HrPOPK1* (Sasakura et al., 2002) is also particularly CAC-rich (containing a cluster of 9 out of 20 CACs in a stretch of 400 bases). Our hope is that this type of sequence analysis may lead to prediction of additional *postplasmic/PEMs*, particularly those of low abundance. More sophisticated computational analysis, coupled with experimental testing of small UTR regions for localization and identification of RNA-binding proteins, will be needed before the rules for RNA localization in ascidians can be understood and compared with those being defined in *Xenopus* and *Drosophila* oocytes and in somatic cells (Kloc et al., 2002).

**Cytoskeleton-driven reorganizations in the zygote and then unequal cleavages segregate *postplasmic/PEM* RNAs in posterior micromeres (see Figure 1)**

Since Conklin, the term 'myoplasm' has been used loosely to designate the entire region extending from the surface (plasma membrane) to the internal cytoplasmic boundary of the mitochondria-rich layer. This peripheral layer is 6–8  $\mu\text{m}$  thick in *Ciona* and *Pballusia* eggs (100–120  $\mu\text{m}$  in diameter), and 15–20  $\mu\text{m}$  in the larger *Halocynthia* egg (350  $\mu\text{m}$  in diameter). *postplasmic/PEM* RNAs (*Type I*) have been originally described as localized in the myoplasm (Yoshida et al., 1996; 1997; Nishikata et al., 2001; Nakamura et al., 2003). Before cleavage this broadly defined myoplasm domain is seen at low resolution as a posterior crescent. It is inherited by the blastomeres which differentiate into primary muscle cells (B7.4, B7.5, B7.8) whereas *postplasmic/PEM* RNAs concentrated in the CAB region (see below) inherited by the posterior-most small blastomeres (B7.6). It has been suggested that the myoplasm is rich in intermediate filaments recognized by a particular antibody (NN18; see Swalla et al., 1991), but in fact this antibody recognizes mitochondrial ATPase (T. Nishikata, personal communication). The specific cytoskeletal composition of the myoplasm still needs to be properly defined.

It is clear that the loose use of the term myoplasm which has prevailed is inadequate to understand *postplasmic/PEM* RNA localizations; therefore, a better description of the substructure of the oocyte cortex and subcortex is required. High resolution analysis shows that the peripheral region of *Pballusia*, *Ciona* and *Halocynthia* oocytes is, in fact, made of two main layers (Roegiers et al., 1999; Sardet et al., 1992; Speksnijder et al., 1993, C. Sardet and F. Prodon, unpublished data) (see Figure 1). (1) The cER-rich cortical domain, a thin (0.5  $\mu\text{m}$ ) cortical layer underlying the plasma membrane made of a monolayer network cER, microfilaments, microtubules and macromolecular complexes associated with these networks. It is this cortical layer which can be obtained attached to the plasma membrane as isolated cortices (see Sardet et al., 2002; Sardet et al., 2003). At least *Hr-PEM1*, *Ci-PEM1*, and *Hr-macho1* colocalize with this cER network and we now call their association the cER/mRNA domain (Sardet et al., 2003; F. Prodon, P. Dru and C. Sardet, unpublished data).

(2) The mitochondria-rich subcortical domain (5–7- $\mu\text{m}$ -thick in *Ciona* and *Pballusia*, 15–20- $\mu\text{m}$ -thick in *Halocynthia*) rich in tightly packed mitochondria embedding vesicles (pigmented in some species) and traversed by cytoplasmic veins containing ER strands from the deeper cytoplasm and in continuity with cER. It is this mitochondria-rich domain which is inherited by blastomeres differentiating into primary muscle cells (B7.4/B7.5/B7.8; see Figure 1) that ought to be called myoplasm (to avoid confusion we call it the mitochondria-rich myoplasm).

These two peripheral layers (cER-rich domain and mitochondria-rich myoplasm domain) are distributed as an increasing gradient along the animal vegetal axis of the oocyte (Sardet et al., 1992; Speksnijder et al., 1993). This polarization is apparently acquired during meiotic maturation of the oocyte (F. Prodon and C. Sardet, unpublished data). The cER-rich domain and mitochondria-rich myoplasm domain are reorganized and relocated in two major phases (including several subphases) after fertilization which are schematized in Figure 1 (Chiba et al., 2004; Roegiers et al., 1999). The 2 peripheral domains (cER-rich and mitochondria-rich myoplasm) are then inherited by two vegetal posterior blastomeres at the 8-cell stage. The cER-rich cortical domain and mitochondria-rich myoplasm segregate during three unequal cleavages between the 8- and 64-cell stage. As shown in Figure 1, the *postplasmic/PEM* RNAs are inherited with the cER-rich CAB by the most posterior micromeres (B7.6). The mitochondria-rich myoplasm is principally inherited by the muscle precursor blastomeres (B7.4/B7.5/B7.8).

The unequal cleavages are thought to be mediated by a peculiar cortical structure which corresponds to the location of the PEM where *postplasmic/PEM* RNAs accumulate (Figures 2f–2h, 2r and 2t). This macroscopic cortical structure has been called the CAB (Nishida, 2002a; Nishikata et al., 1999). Using high resolution *in situ* hybridization and isolated cortices, we have shown that *Hr-PEM1* and *Hr-macho1* are concentrated in the CAB together with the cER-rich domain (Figure 2o; Sardet et al., 2003). We have suggested that these mRNAs translocate with cER after fertilization and that the cER/mRNA domain is a major precursor of the CAB. Recent experiments show this also to be true for *Ci-PEM1* (F. Prodon, C. Sardet and P. Dru, unpublished data) but we have not yet examined other *postplasmic/PEM* RNAs.

### Perspectives and speculation on the role of *postplasmic/PEM* RNAs

Considering that ascidian oocytes, zygotes and embryos are made of a juxtaposition of cytoplasmic, cortical and subcortical domains with distinct compositions in organelles and cytoskeletal elements, it is not surprising that specific mRNAs associated with these localized domains will be inherited and segregated in different blastomeres. In terms of differentiation and morphogenesis the most interesting domains and RNAs are clearly those located in the periphery of the egg, where at least five determinants have been located by micro-ablations (reviewed in Nishida, 1997). It remains to be seen whether the yet-undefined determinants for ectoderm, endoderm, unequal cleavage and gastrulation will turn out to be cortical mRNAs as is the case for the muscle cell determinant *macho1*.

The mechanisms of maternal cortical mRNA localization are intensely studied and are starting to be understood in *Drosophila* and *Xenopus* oocytes, where regions of 3'UTR associate with particular RNA-binding protein complexes that form and are moved by the cytoskeletal machinery, anchored to organelles (e.g. ER) or cytoplasmic granules (e.g. germ plasm) (Kloc et al., 2002). In contrast in ascidians, we know nothing of the mechanisms used by *postplasmic/PEM* mRNAs to reach the oocyte cortex and if there are late and early pathways of localization and if diffusion-entrapment mechanisms are used during oogenesis as recently shown in *Xenopus* (Chang et al., 2004). It is clear however that in *Halocynthia* zygotes (Sardet et al., 2003) and *Ciona* (F. Prodon, P. Dru and C. Sardet, unpublished data) some *postplasmic/PEM* mRNAs are moved together with the cER after fertilization. Our working hypothesis is that these RNAs are associated with the rough cER network via RNP particles containing RNA-binding proteins such as have been described in mammalian neurons or *Xenopus* oocytes (Belanger et al., 2003; Ohashi et al., 2002; Allison et al., 2004).

The study of the storage and translational control of mRNAs and the role of RNPs has just started in ascidians (Tanaka et al., 2004). One handicap is that for lack of proper antibodies the expression or localization patterns of the proteins coded by *postplasmic/PEM* RNAs are unknown. One interesting exception is Cs-PEM3 for which an antibody was obtained which shows that the PEM3 protein occupies a much larger region of the posterior pole of the 8-cell stage embryo

(including animal posterior blastomere) than that occupied by PEM3 mRNA, which is restricted to the CAB region (see Figure 5B in Satou, 1999). This is reminiscent of the classical situation described for *Bicoid* RNA distributed in a steep cortical gradient generating a shallower Bicoid protein gradient in the anterior region of the fly oocyte (Nusslein-Volhard, 2004). It is very likely that proteins coded by maternal *postplasmic/PEM* mRNAs and in particular the muscle determinant *macho1* are synthesized before the 16-cell stage, since *macho1* must exert its effect in muscle precursor blastomeres that do not inherit the posterior-most cER/mRNA domain concentrated in the CAB (Kobayashi et al., 2003). That translation occurs before the 16-cell stage is also supported by a recent analysis of polyribosomes and of a Y-box protein (CiYB1) and its mRNA thought to be involved in storage and translational control of mRNAs in oocytes and which have been shown to be partially colocalized with *postplasmic/PEM* mRNAs (Tanaka et al., 2004).

Of all *postplasmic/PEM* RNAs, *macho1* has attracted most attention. Recent experiments by Nishida and colleagues using isolated and reassociated vegetal blastomeres depleted or not of *macho1* (using morpholinos) show that its role is in fact more extensive than that of a maternal determinant for primary muscle cell differentiation (Kondoh et al., 2003). The presence of *macho1* in a cortical location and the inferred (but not yet demonstrated) wider distribution of Macho1 protein patterns the differentiation of the marginal zone. The marginal zone in the vegetal hemisphere surrounds a central endodermal mass and gives rise to mesodermal tissues: primary muscle (cell-autonomous differentiation) as well as notochord, mesenchyme, trunk ventral and lateral cells which result from inductions from endoderm precursors at the 32–64-cell stages. Responsiveness of presumptive notochord and mesenchyme to induction involves fibroblast growth factor-like factors and the Ras-MAPK signalling pathway and depends also on the presence of maternally-inherited factors (and particularly the product of *macho1*) in the posterior vegetal cytoplasm (but not in anterior) (Kobayashi et al., 2003). It was thought until recently that like primary muscle cells, endoderm cell differentiation in ascidians was due solely to the inheritance of some, as yet undefined, maternal determinants situated in the vegetal periphery of the egg at the time of cleavage.

However, recent experiments based on the isolation of posterior endoderm precursor blastomeres show that they develop into muscle cells (a default state) rather than endoderm (an induced state without cell interactions). Suppression of *macho1* function by signalling from neighbouring blastomeres is in fact needed to allow endoderm differentiation (Kobayashi et al., 2003; Kondoh et al., 2003). These observations have led to the notion that patterning of the ascidian embryo results from an interplay of posteriorly localized factors (*macho1* and probably other *postplasmic/PEM* RNAs) acting as a source of cytoplasmic maternally derived factors whose influences are modulated by extrinsic induction signals. The nature of the interaction between cytoplasmic factors of maternal origin and oriented signals coming from neighbouring blastomeres is a matter of speculation for the moment. The fact that this involves a small number of unduplicated genes acting at the single cell level makes ascidian embryos an attractive model to study the role of maternally-localized mRNAs in polarization, differentiation and morphogenesis (Imai et al., 2004; Satoh et al., 2003; Nishida et al., 2002a, 2002b).

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