The maternal control in the embryonic development of zebrafish

Andrea Miccoli[a], Luisa Dalla Valle[b,] Oliana Carnevali[a,*]

[a] Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy

[b] Department of Biology, University of Padova, Padova, Italy

Abstract

The maternal control directing the very first hours of life is of pivotal importance for ensuring proper development to the growing embryo. Thanks to the finely regulated inheritance of maternal factors including mRNAs and proteins produced during oogenesis and stored into the mature oocyte, the embryo is sustained throughout the so-called maternal-to-zygotic transition, a period in development characterized by a species-specific length in time, during which critical biological changes regarding cell cycle and zygotic transcriptional activation occur.

In order not to provoke any kind of persistent damage, the process must be delicately balanced. Surprisingly, our knowledge as to the possible effects of beneficial bacteria regarding the modulation of the quality and/or quantity of both maternally-supplied and zygotically-transcribed mRNAs, is very limited. To date, only one group has investigated the consequences of the parentally-supplied *Lactobacillus rhamnosus* on the storage of mRNAs into mature oocytes, leading to an altered maternal control process in the F1 generation. Particular attention was called on the monitoring of several biomarkers involved in autophagy, apoptosis and axis patterning, while data on miRNA generation and pluripotency maintenance are herein presented for the first time, and can assist in laying the ground for further investigations in this field.

In this review, the reader is supplied with the current knowledge on the above-mentioned biological process, first by drawing the general background and then by emphasizing the most important findings that have highlighted their focal role in normal animal development.

1. Introduction

The successfulness of biological processes occurring during the earliest steps of the embryonic development of zebrafish (Pelegri, 2003), as well as of all vertebrate and invertebrate organisms (e.g. Drosophila – St Johnston and Nüsslein-Volhard, 1992; Caenorhabditis elegans – Kemphues and Strome, 1997; ascidians – Nishida, 2005; *Xenopus* – Heasman, 1997) strictly depends on maternal factors (i.e. mRNAs, proteins or any other biomolecule) originated during oogenesis (Babin et al., 2007). Their amount and localization into oocytes influence precise embryonic developmental schemes and determine their functions (Babin et al., 2007). Maternal gene products regulate all aspects of both oocyte and embryonic development. These include oocyte maturation (Lee et al., 2014), fertilization and transitions between meiotic and mitotic cell cycles (Marlow, 2010), cell fate and patterning (Tadros and Lipshitz, 2009), and processes related to cellular metabolism (Pelegri, 2003) among others.

Howley and Ho (2000), expanding previous research by Bally- Cuif et al. (1998), showed the localization of b-catenin, pou-2, cyclin B, vasa and dazl, used as references, in zebrafish ovaries by whole mount in situ hybridization. By this study maternal mRNAs were classified into four classes: (i) ubiquitously distributed, (ii) localized at the animal pole, (iii) at the vegetal pole and (iv) in the cortex. Such localization was established at the end of the vitellogenesis, when the nucleus moves towards the animal pole (Babin et al., 2007).

Early embryo development is characterized by synchronous and rapid cleavages lacking the G phase that generate a large population of zygotic blastomers (Marlow, 2010) during the maternal-to-zygotic transition (MZT). During this period, maternal transcripts begin to be depleted, transcription is initiated and cell cycle changes, and towards its end cells become susceptible to apoptosis (Langley et al., 2014; Stack and Newport, 1997; Tadros and Lipshitz, 2009). This stage also includes the midblastula transition (MBT), a precise developmental point, at which the cell cycle length increases and loses synchrony, and large-scale zygotic transcription is activated (Kane and Kimmel, 1993). Following MBT, cells acquire mobility and can manifest apoptosis in response to DNA damages caused by cleavage replications (Ikegami et al., 1997; Kane and Kimmel, 1993; Newport and Kirschner, 1982a; Stack and Newport, 1997).

Two models have tried to explain distinct features of the transition between the maternal and zygotic functions: the nucleocytoplasmic (N/C) ratio model (Newport and Kirschner, 1982a,b) and the maternal clock model (Howe et al., 1995). The former proposes the presence of a transcription repressor in the early embryo, whose titration depends on subsequent cell divisions and the consequently increasing ratio of nucleus (or DNA) to cytoplasm; the latter suggests that a molecular clock responsible for regulating the cell processes that happen within the MZT is set into motion by fertilization.

Both models were recently integrated into a more detailed mechanism that aims at thoroughly explaining how zygotic transcription is initiated (Langley et al., 2014). According to it, subsequent cell divisions first titrate out the transcription repressor that holds genomic DNA into a repressed state. After fertilization, maternal mRNAs maintained in an inactive state by the binding of proteins to specific regions at their 3'-UTR (Groisman et al., 2002; Harvey et al., 2013; Mendez and Richter, 2001) are polyadenylated and afterwards translated, in order to accumulate the correct repertoire of transcription factors. These are available at the 128-cell stage in zebrafish, but transcription is still prevented from beginning until genomic DNA switches into a compatible state. Chromatin regulation was suggested to control the timing of gene activation during the MZT (Akkers et al., 2009; Chen et al., 2013; Lindeman et al., 2011; Prioleau et al., 1994; Schuettengruber et al., 2009; Vastenhouw et al., 2010), but it is still controversial whether methylation at the lysine 4 or 27 (H3K4me3 and H3K27me3) plays a role. These marks are normally associated with transcriptionally active and

repressed genes, respectively. Some authors (Barski et al., 2007; Chen et al., 2013; Vastenhouw et al., 2010) did not find them either in the zebrafish or Drosophila until zygotic transcription is initiated, whereas others (Jiang et al., 2013; Potok et al., 2013) recently evidenced the tight regulation of methylation by early zebrafish embryo whole-genome bisulfite sequencing. Akkers et al. (2009), Lindeman et al. (2011) and Vastenhouw et al. (2010) reported the occurrence of H3K4me3 across promoters of early embryonic genes, while H3K27me3 is associated to genes encoding specific developmental functions.

Expectedly, the DNA methyltransferase Dnmt1 was also suggested to control transcriptional repression in the early embryo, as its MO-knockdown resulted in the premature expression of some genes in *Xenopus* (Dunican et al., 2008; Stancheva and Median, 2000). Through knockdown and knockout experiments, Dnmt1's role in zebrafish embryos was found crucial for proper differentiation of the intestine, exocrine pancreas, and retina (Rai et al., 2006), as well as for the development and maintenance of lens (Tittle et al., 2011). Its role is strongly linked to transcriptional activity, as inactive Dnmt1 altered gene expression. Deleterious effects could be rescued either by active zebrafish or human DNMT1 (Rai et al., 2006).

The advent of microarrays and high-throughput RNA sequencing technologies have revolutionized the field. On one hand, they have allowed a deeper understanding of the maternal contribution's extent; on the other, they unveiled the challenge of detecting transcriptionally-active genes when numeric discrepancy between maternal and zygotic copies is so great (Lee et al., 2014) or when degradation of maternal copies occurs concurrently with de novo transcription, in fact canceling the signal (De Renzis et al., 2007). RNAs inherited by the mothers account for 40–75% of all functional genes. Such number is far higher than that of zygotic transcripts (Tadros and Lipshitz, 2009; Wang et al., 2004; Wei et al., 2006), and it amounts to 60–70% of all mRNA molecules at zebrafish's peak zygotic expression (Lee et al., 2013). In order to obtain a more accurate assessment of the expression, several methods were applied to the zebrafish model. Rothstein et al. (1992) depleted the maternal contribution by means of subtractive hybridization techniques; Newport and Kirschner (1982a) discriminated zygotic contribution by labeling it with modified ribonucleotides; Hamatani et al. (2004) under-represented the zygotic contribution by applying RNA polymerase-inhibiting a-amanitin and actino-mycin; taking advantage of the RNAseq techniques' ever- increasing sensibility, it was possible to distinguish zygotic transcripts from maternal ones by specific alternative splicing (Aanes et al., 2013), transcription start sites (TSSs) (Haberle et al., 2014; Nepal et al., 2013), and polyadenylation (Ulitsky et al., 2012).

The timing of genome activation varies according to the experimental model: as a general rule, the number of cell cycles required for transcriptional initiation is higher in lower vertebrates than in mammals. Zebrafish embryos perform a number of cell divisions ranging between 7 and 9 (64-cell to 256-cell stages) (Aanes et al., 2011; Harvey et al., 2013; Heyn et al., 2014; Lee et al., 2013), and 12 (Kane and Kimmel, 1993; Newport and Kirschner, 1982a) before zygotic genome is activated. Transcripts increase from several hundreds (Heyn et al., 2014) to several thousands prior to gastrulation (Harvey et al., 2013; Lee et al., 2013). Since embryonic genome is held inactive, the mitotic cycles preceding transcription only rely upon maternal products as well as on the contribution from sperm (Dae and Roy, 2006; Yabe et al., 2007), although the latter was assumed as minimal by Sawicki and colleagues (1981).

Despite on *Xenopus* rather than zebrafish, the study of Collart and colleagues (2014) exemplified the behavior of genes during the first hours of life through high-resolution expression profiling. Authors revealed that the onset of zygotic gene expression is not sudden, rather it proceeds in the form of waves (Tadros and Lipshitz, 2009). Three waves of gene activity involving maternal transcripts' polyadenylation, zygotic transcription and a final, shorter, post-MBT wave were found. A broader network of early- expressed genes is needed for transcriptional regulation, stem cell maintenance and axis patterning (Langley et al., 2014), and indicate both the multifactorial

complexity of the embryonic development and the delicate regulation to which the maternal control must be subjected in order not to induce any damages.

The ability of the gut microbiota to communicate with the brain and to modulate behavior by intervening at, among others, the neuroendocrine level, is a concept that has been emerging domineeringly in the last years, so that the actual existence of a microbiome-gut-brain axis is being recognized (Cryan and O'Mahony, 2011). Nonetheless, very little research has focused on how the maternal control can be possibly modulated by the administration of beneficial bacteria that affect the natural gut microbiota by establishing new ecological relations. The Lactic Acid Bacteria (LAB) were already demonstrated to be responsible for providing benefits to normal biological processes such as immune system (e.g. Balcázar et al., 2007; Verschuere et al., 2000), nutrient metabolism (e.g. Falcinelli et al., 2015, 2016; Suzer et al., 2008), growth (e.g. Carnevali et al., 2006), stress tolerance (e.g. Gioacchini et al., 2014; Rollo et al., 2006), bone calcification (e.g. Avella et al., 2012; Maradonna et al., 2013), development and reproduction (e.g. Carnevali et al., 2013; Castex et al., 2008; Gioacchini et al., 2012).

In our laboratory, we called our attention on the subject and we investigated whether a specific supplementation of the LAB *Lactobacillus rhamnosus* to parental fish could influence the quality and/ or the quantity of both maternally- and zygotically-controlled transcripts in the F1 generation (Miccoli et al., 2015). We explicitly focused on autophagy, apoptosis and axis patterning by monitoring the temporal and spatial expression patterns of such key developmental processes' well-established biomarkers. Additional results regarding the effects of *L. rhamnosus* on miRNA processing and stem cell pluripotency will be presented for the first time.

We herein review the current knowledge about the above- mentioned key biological activities playing a central role during development and report novel findings relating probiotic supplementation, miRNA processing and stem-cell pluripotency signals.

2. Autophagy-related biomarkers

Autophagy is an intracellular self-degradative catabolic pathway useful for recycling unnecessary/harmful cytoplasmic constituents through the lysosomes via a double membrane vesicle named autophagosome. The multiple targets are long-lived, misfolded or aggregated proteins, damaged organelles such as mitochondria, ribosomes or endoplasmic reticulum, and pathogens (Funderburk et al., 2010; Glick et al., 2010). With such proteolytic process, the organism maintains cellular homeostasis and balances sources of energy during development and in response to both extracellular (e.g., nutrients, oxygen availability, overcrowding, and temperature) and intracellular stressors (Klionsky and Emr, 2000; Levine and Klionsky, 2004).

Three variants of autophagy exist – macroautophagy, microautophagy, and chaperone-mediated autophagy (Mizushima and Komatsu, 2011). These differ in terms of the pathway used to deliver the material to be degraded to the lysosome compartment, which can either be systematic and autophagosome-mediated (Masaki et al., 1987) or occasional and directly involving the lysosomal membrane through invagination (Marzella et al., 1981). In all cases, they share a common degradative compartment and process, which ultimately leads to the redistribution of the recycled substances (Levine and Klionsky, 2004). The term autophagy usually refers to the macroautophagy process.

A major molecular advancement regarding autophagy came from the discovery of the yeast autophagic machinery components, the AuTophagy-related (ATG) Genes. ATG1, ATG5, ATG6 and ATG13 were firstly discovered and cloned by Prof. Ohsumi's research group (2006). More than thirty-seven Atg proteins are known and reviewed in terms of function (Mizushima et al., 2011) and nomenclature (Klionsky et al., 2003).

The fundamental mechanism of autophagy is evolutionary conserved, as ATG homologs were discovered in human (Mizushima et al., 1998). Authors identified hAtg12, hAtg5 and the conjugation between them, similarly to the yeast's counterparts. The additional discovery of Apg16L-mouse homolog of Atg16- (Mizushima et al., 2003), confirmed the widespread conservation of the system throughout eukaryotes.

The dynamics of autophagy induction have been unveiled and consist in a multi-factorial phosphorylation-inductive interactions among Atg proteins (e.g. Ohsumi, 2014), overall resulting in the translocation of protein complexes at the endoplasmic reticulum and the formation of the autophagosome, first, and the autolysosome (e.g. Klionsky et al., 2014), thereafter. Among the pivotal Atg proteins, Beclin 1 must be mentioned. Such direct interactor of the Bcl-2 protein was discovered in late 1990s (Liang et al., 1999). The mammalian gene and the encoded protein have a structural similarity with the yeast apg6/vps30 (Liang et al., 1999), reason for which it was included into the Atg family and is also known as Atg6 (Klionsky et al., 2003). Such evidences demonstrated that Beclin 1 is highly conserved in eukaryotes.

Beclin 1 is part of the phosphatidylinositol 3-kinase class III (PI3KIII) complex. Once activated, the complex leads to the generation of phosphatidylinositol 3-phosphate (PI3P) which eventually recruits Atg proteins involved in the autophagosome biogenesis (Kihara et al., 2001). In order to test for Beclin 1's role, Yue and colleagues (2003) generated Beclin 1-knockout mice and found that loss of this protein leads to an early death during embryogenesis, demonstrating Beclin 1's vital importance in such developmental phase. Beclin 1 constitutes the platform of the multifactorial autophagic signaling, as evidenced by the presence of several domains in its 60-kDa protein structure that enables multiple protein interactions (Erlich et al., 2007; Furuya et al., 2014; Pattingre et al., 2005). For details, the Beclin 1-Vps34 interaction and all the mammalian associated binding partners have been thoroughly reviewed by Funderburk and co-workers (2010).

While the structure and functions of Beclin 1 are well- understood, little is known about its regulation (Wirawan et al., 2012). Transcriptionally, BECN1 possesses miRNA30a binding sites in its 3' -UTR

(Zhu et al., 2009), while the promoter region and the second intron can be hypermethylated and possibly subjected to a decreased expression (Li et al., 2010). Post-translationally, Beclin 1 can be proteolytically cleaved by caspases (Luo and Rubinsztein, 2009): the resulting fragments were defined cytotoxic (Wirawan et al., 2012), as they do not only lose the ability to induce autophagy, but also serve as a positive feedback loop stimulating cell death (Luo and Rubinsztein, 2009; Wirawan et al., 2010). Other than the well-characterized roles, Beclin 1 has autophagy- independent functions. For instances, it is involved in the endocytic pathway in macrophages (Sanjuan et al., 2007) and apoptotic cell clearance (Qu et al., 2007). Beclin 1's activity can also be regulated by the interaction with other proteins, as indicated by the binding with Bcl-2 and Bcl-xL at its N-terminal BH3 domain (Erlich et al., 2007; Maiuri et al., 2007a; Pattingre et al., 2005). These bindings suppress autophagy, indicating that such Bcl-2 family members have other roles in addition to the anti-apoptotic one, which would be anyway maintained (Ciechomska et al., 2009). Beclin 1-dependent autophagy can also be regulated by the peptide's sub-cellular localization: under physiological condition, autophagy is prevented from being triggered because of the Ambra1 binding to the Beclin 1-PI3KIII complex at microtubules (Di Bartolomeo et al., 2010).

Ambra1 protein (Activating Molecule in Beclin-1-Regulated Autophagy) in fact has the greatest effect as it holds the autophagic key complex in place until the triggering stimulus is received. Upon autophagy induction, Ambra1 receives an ULK1-mediated phosphorylation, which causes its releases from the dynein chains and allows the translocation of the complex to the autophagosome forming spot, the endoplasmic reticulum. Other than receiving a phosphorylation by ULK1, it was recently found that Ambra1 itself is able to exert a regulatory control on the activity of ULK1 through ubiquitylation and stabilization, in fact positively self-feedbacking. This suggests that Ambra1 might have a broader role in affecting the successfulness of autophagy (Nazio et al., 2013), also by coordinating several other process such as selective mitochondria removal and cell cycle down-regulation or by being subjected to post-translational modifications including caspase cleavage (Cianfanelli et al., 2015).

A study noted that autophagy ''is enhanced in cells undergoing remodeling in the course of differentiation or other induced changes, as in newborn kidney, lung, intestine, fetal duodenum, metamorphosing insect salivary glands, regressing Mullerian ducts, amphibian erythrocytes, keratinizing skin, and rat prostate after castration" (Deter and De Duve, 1967). Autophagy is hence involved in cellular architectural changes that specifically occur during differentiation and development. Indeed, Ambra1 is crucial during vertebrate embryogenesis: during mouse development Ambra1 is highly expressed in neuroepithelium and its knockout in mouse leads to early embryonic lethality, exencephaly and imperfect neural tube closure (Fimia et al., 2007). Accordingly, knockdown by translation-blocking morpholino showed that the two Ambra1 proteins, produced by the two paralogous genes identified and characterized in zebrafish, are both required during development as their silencing results in developmental abnormalities consisting in body growth delay, curved shape, hemorrhagic pericardial cavity and neural tube defects (Benato et al., 2013). Ambra1 proteins are also crucial for the correct development and morphogenesis of skeletal muscle in zebrafish. Noteworthy, muscle defects were rescued after co-injection of human AMBRA1 mRNA, pointing out the conservation of Ambra1 functions through evolutionary times (Skobo et al., 2014). Moreover, the cloning and characterization of Ambra1 in the tunicate Botryllus schlosseri has recently demonstrated that Ambra1 is an ancient gene, having evolved distinctly at least before the radiation of Bilateria (Gasparini et al., 2016).

Autophagy defects lead to various neurodegenerative and lysosomal-related diseases, and to oncogenesis and cancer progression (Mizushima and Komatsu, 2011). For instance, MO- induced ablation of Ambra1 caused severe developmental issues, neural abnormalities, decreased viability, reduced autophagy and increased apoptosis (Benato et al., 2013). Accordingly, Ambra1's functional deficiency in mice is typically accompanied by the appearance of a large number of apoptotic cells

(Cecconi et al., 2008; Fimia et al., 2007). Reduced levels of Ambra1 cause increased susceptibility to different apoptotic stimuli (Pagliarini et al., 2012).

Maiuri et al. (2007b) reported the complex relationship between autophagy and apoptosis, stating that proteins that are able to induce cell death can also induce autophagy and vice versa. The anti-apoptotic factor Bcl-2 acts as a factor in such crosstalk. A dynamic mitochondrial interaction between Ambra1 and Bcl-2 regulates both Beclin-1-dependent autophagy and apoptosis. In physiological conditions, Ambra1 is prevented from associating with the Beclin1-PI3KIII complex; if apoptosis is induced, Bcl-2 releases Ambra1, which is then possibly degraded by caspases (Strappazzon et al., 2011).

Ambra1a1 and 1b paralogous genes, as well as becn1 and lc3 can be all influenced by the supplementation of beneficial bacteria, such as *L. rhamnosus*, which are able to alter zebrafish's own microbiota. The administration of the probiotic *L. rhamnosus* on zebrafish adult fish produced evident effects on ovarian autophagic and apoptotic processes (Gioacchini et al., 2013) as well as on both maternal and zygotic levels of such autophagy-related signals (Miccoli et al., 2015). In embryos from probiotic-treated fish ranging from 0 h post-fertilization (hpf) to 4 hpf they are present in lower relative amounts than controls' (Fig. 1A–D). All mentioned signals are maternal factors: ambra1a and becn1 were replaced by zygotic messages after 8 hpf, while ambra1b and lc3 maintained themselves to expression levels as high as those present at the

75%-epiboly stage. It must be kept in mind that zebrafish does not show any evidence of autophagy until 32–48 hpf (He et al., 2009). Therefore, as previously illustrated, it is likely that the transcripts in question have an active role in developmental processes other than autophagy, and their levels, despite lower than control's, were still able to ensure normal development.

3. Apoptosis-related biomarkers

Apoptosis is a genetically encoded program of cell death involved in many biological process and functions: organisms carry it out to ensure normal cell turnover, proper embryonic development, correct functioning of the immune system and tissue homeostasis (Elmore, 2007) but can adopt it also as a defense mechanism against harmful agents (Norbury and Hickson, 2001). The term was coined by Kerr et al. (1972), although some concepts on apoptotic components had been previously derived by the pioneering researches of Walther Flemming, who began investigating the dividing animal cell's mechanisms at the end of the 19th century. His interests and discoveries enlightened the path towards 'uncontrolled' growth of cancer and cell-cycle regulation (Paweletz, 2001).

Vertebrates have two apoptotic signaling mechanisms: the ancient cell-intrinsic (also known as mitochondrial) and the more recent cell-extrinsic (or death receptor) pathways. DNA damage or endoplasmic reticulum stress, growth factor withdrawal and chemotherapeutic drugs are all examples of factors that trigger the cell-intrinsic pathway's initiator caspase 9, which subsequently hands the regulation to the regulatory Bcl-2 gene family (Cory and Adams, 2002; Youle and Strasser, 2008). Such family comprises both pro-apoptotic (Bak, Bax and Bok-commonly referred to as effectors since they cause mitochondrial outer membrane permeabilization-, Bid, Bim, Bad, Bik, Bmf, Bnip3, Hrk, Noxa and PUMA) and anti-apoptotic proteins (Bcl-2, Bcl-w, Bcl-xl, A1 and Mcl-1). The latter differ in the presence and the displayed number of a-helical BCL-2 homology (BH) domains designated as BH1, BH2, BH3 and BH4 (Tait and Green, 2010). The extrinsic pathway is instead pivotal for the mammalian's immune system (e.g. Chun et al., 2002; Strasser et al., 2009; Chowdhury et al., 2008), but it is unclear whether it plays a similar role in zebrafish, in which T and B cells do not develop until 3 dpf or later (Trede et al., 2004). Investigations so far have been complicated by the fact that the TUNEL assay is impractical after 36–48 hpf because of a strong background staining (Rodriguez and Driever, 1997). The recruitment of the FAS-associated death domain protein (FADD)

adaptor molecules and caspases 8 and 10 is initiated by the ligation of death ligands to their cognate death receptors (Wilson et al., 2009).

The two pathways share some common peculiarities: both are highly complex and energetically demanding (Elmore, 2007) and converge at the level of caspase 3 and 7, defined executioners (Tait and Green, 2010). The identification of the caspase 8- mediated cleavage of Bid [BCL-2 homology 3 (BH3)-interacting domain] provided a molecular basis of the strong cross talk existing between the two apoptotic networks (Igney and Krammer, 2002). The molecular mechanisms of apoptosis and many of the genes that have a role in the killing and engulfment processes are extremely well-conserved among all metazoans (Cole and Ross, 2001; Metzstein et al., 1998; Yamashita, 2003). Orthologs of the Bcl-2 family have been described in zebrafish (Kratz et al., 2006) and its anti-apoptotic members show structural and functional conservation with mammals (Jette et al., 2008), thus validating *Danio rerio* as a model to expand our knowledge on the molecular mechanisms regulating apoptosis. As an example, zebrafish effector caspases can cleave many of the same protein substrates known in mammals, including PARP and 14 novel human caspase 3 substrates (Valencia et al., 2007).

The organism's well-being is ensured only if apoptosis is subjected to a fine regulation during cell growth and differentiation (e.g. Ellis et al., 1991); if mis- or deregulated (either too little or too much cell death), it causes diverse embryo abnormalities and pathologies such as neurodegenerative diseases, ischemic damages, autoimmune disorders and many types of cancers (Tait and Green, 2010). Normal development also relies on a careful balance among proliferation, differentiation, and death by apoptosis, with cells being continuously over-produced and eliminated. Although apparently wasteful energy-wise, this is a common feature of metazoan development and occurs in species ranging from nematodes to humans (Meier et al., 2000; Penaloza et al., 2006).

Negron and Lockshin (2004) and others (Ikegami et al., 1999, 1997), found that zebrafish embryos do not manifest apoptosis in the first hours of life ranging between fertilization and the maternal-to-zygotic transition. Alike observations were reported for C. elegans, Drosophila melanogaster and *Xenopus* laevis (Greenwood and Gautier, 2005). However, Yabu et al. (2001), while assessing by Northern blot the expression of zebrafish caspase 3 transcripts after 1 (four-cell), 3 (1000-cell), 6 (shield), 12 (one-somite), 24 (pharyngula period) and 48 h (hatching period), detected its presence in all developmental stages. Keeping in mind the zygotic genome quiescence before ZGA, caspase 3 mRNA in fertilized eggs was proposed to be maternally-supplied. Embryos treated with cell death-inducers camptothecin, cycloheximide, nocodazole, or staurosporine (Belmokhtar et al., 2001; Endo et al., 2010; Morris and Geller, 1996; Tang et al., 1999) prior to MBT show an arrest of cell proliferation but survive until the mid-gastrula stage (about 7 hpf), at which point they undergo rapid and synchronous cell death. Therefore, the acquisition of apoptotic competence was proposed to be post-translationally controlled, similarly to what happens in X. laevis pre-gastrula stage embryos (Hensey and Gautier, 1997). Mammalian embryos, in contrast, were shown to display apoptotic cells as early as the 16-cell stage (Penaloza et al., 2006).

Despite the term apoptosis could imply a negative acceptation, its contribution is essential in processes such as cellular differentiation (Abraham and Shaham, 2004) and development (Wang and Lenardo, 2000). Caspases were found to be required for sperm differentiation in Drosophila (Arama et al., 2003). Knockdown experiments carried out on mouse caspase 8 caused death because of the insurgence of cardiac muscles anatomy defects and circulatory failure (Kang et al., 2004). Kuida et al. (1996) and Hakem et al. (1998), through similar knockdown approaches, found that the survival rates of caspase 3 and 9 deficient mouse embryos were extremely low because of significant defects in the brain anatomical structures caused by a dramatic reduction of the apoptotic process in rapidly dividing neuroepithelial cells.

In addition, Miccoli et al. (2015), having assessed the expression levels of caspase 3, bax and bcl2 after parental *L. rhamnosus* administration, found that apoptotic-related biomarkers were promoted

in their gene expression over autophagic ones, as illustrated by overall higher maternal and zygotic mRNA abundances of signals belonging to both cell death pathways (exception made for bcl2, which is an anti-apoptotic signal) (Fig. 1E–G). Such results, obtained by q-PCR, were confirmed by TUNEL assay, as shown by the higher and lower abundance of apoptotic nuclei found at 12 and 24 hpf, respectively, in the probiotic-treated group (Fig. 1J). The results demonstrated the probiotic's ability to boost zebrafish development and modulate the storage of maternal mRNAs into the oocytes, in fact favoring biomarkers of the apoptotic process in embryos belonging to the treated group, especially in the first hours of life.

Taken together, these examples indicate the remarkable role of caspases, particularly Caspase 3, which lies beneath normal development and embryogenesis (Yabu et al., 2001), since cellular degradation and proliferation are at the basis of differentiation and development (Oppenheim, 1991).

4. Axis patterning signals

During early vertebrate life, the developing embryo performs crucial processes, upon which survival and well-being at future stages depend. Some of the embryogenesis' most important steps are the determination and patterning of the anteroposterior (A–P), dorsoventral (D–V), and left–right (L–R) axis.

Vertebrate embryonic pattern formation was thought to be triggered at the onset of the gastrulation phase, possibly by a signal from the extraembryonic yolk cell (Koshida et al., 1998; Nikaido et al., 1999). Such hypothesis was confirmed when the removal of the vegetal-most part of the fertilized yolk caused severe ventralization and the lack of dorsal mesoderm as well as of forebrain and midbrain in the zebrafish embryo (Grinblat et al., 1998; Mizuno et al., 1999; Ober and Schulte-Merker, 1999). The yolk was thought to possess a so-called dorsal determinant, whose activity is able to stabilize and translocate the b-catenin transcription factor at the future dorsal side (Schneider et al., 1996). It was hence regarded to as an important source of patterning signals essential for inducing both dorsal and marginal cell fates (Schier, 2001). Similarities among mammalian blastocysts and *Xenopus* oocytes suggested that a polarity exists even earlier in development, prior to the onset of gastrulation. The A–P axis is the first activated pattern, but the current knowledge on the establishment of the dorso-ventral one is the most advanced (Babin et al., 2007).

Transcription factors, ligands and receptors belonging to the Nodal (Imai et al., 2001; Le Good et al., 2005), BMP (Feng and Derynck, 2005), Wnt (Van Es et al., 2003) and Fgf (Thisse and Thisse, 2005) pathways mediate the earliest events in zebrafish development (Le Good et al., 2005). All can be identified as key players that establish morphogenetic gradients for ensuring the patterning processes by diffusing from the early tissues. They control fate near the margin of the yolk, in the ventral side and in the dorsal side, respectively (Chan et al., 2009).

Chordin is a BMP inhibitor, central in the Spemann organizer function (Piccolo et al., 1996; Sasai et al., 1994, 1995). In fact, Chordin-depleted embryos displayed a great expansion of ventral fates (Hammerschmidt et al., 1996), even though a small brain and an axis are still present, presumably because of the action of other anti-BMP signals (Bachiller et al., 2000; Khokha et al., 2005). Yet, if a Chordin-deficient Spemann organizer is transplanted, the ability to induce a second axis or to dorsalize tissues is missed at all (Oelgeschläger et al., 2003). In zebrafish, Chordin is expressed in the dorsal area under the direct regulation of Dharma and acts in concert with Ogon/Sizzled (Muraoka et al., 2006; Wagner and Mullins, 2002), Noggin 1 (Bauer et al., 1998; Dal-Pra et al., 2006), Follistatin (Bauer et al., 1998), and Follistatin-like 1b (Dal-Pra et al., 2006) to inhibit bmp's expression at the dorsal side. Noggin and Follistatin have their expression regulated by BMP2b, Chordin, and Follistatin itself at the mid-gastrula stage (Bauer et al., 1998). Such molecules intervene at the late steps of D–V patterning, in fact refining the effects of Chordin's previous interaction. The direct regulation carried out by Dharma was evidenced by Chordin's blocked/increased expression resulting from mutant and overexpression experiments, respectively (e.g. Sirotkin et al., 2000; Shimizu et al., 2000, 2002; Koos and Ho, 1999). On the other hand, Chordin indirectly enhances the expression of dharma through the inhibition of the expression of BMP- enhanced vent/ved/vox, which normally repress Dharma (Chan et al., 2009). The gene network that determine the D–V axis at the embryo's dorso anterior side also depend on the timing of the embryonic stage. As an example, the scenarios at the early and late gastrula stages are very different in terms of interactions, extent and predominance of signals such as b-Catenin, Chordin, Dharma, Goosecoid, Vent, Ved and Vox. Specifically, the temporal and spatial expressions of sqt, chordin, dkk1 and boz were demonstrated to directly depend upon the activity of the transcriptional activator b-Catenin (Ryu et al., 2001).

The outcome of a proper axis strictly depends on a plethora of contrasting signals interacting with one another with the ultimate aim to define dorsal and ventral regions. Schier (2001) draw what he defined the simplest model of D–V patterning, identifying three consecutive steps. (I) ventral, lateral and posterior development are promoted by BMP and Wnt8, whereas Sqt, Cyc and b-Catenin are

responsible of promoting dorsal development; (II) Boz, Chordin and Dkk1 first promote dorsal development by suppressing Vox, Vent, BMP and Wnt8; (III) Vox and Vent then act as ventral/lateral promoters by repressing Boz, Dkk1, Chordin and Goosecoid. In such a just-apparent straightforward scenario, Chordin fulfills a central role, as its expression at the dorsal region enables the creation of a BMP gradient of activity that decreases from the ventral to the dorsal sides. Yet, the overall effects are due not only on the action of the dorsal center secreting Chordin, Noggin, BMP2 and ADMP – the latter two are growth factors expressed in Spemann's organizer (Inomata et al., 2008), but also on the reaction of the ventral center releasing BMP4 (Fainsod et al., 1994), BMP7 (Reversade et al., 2005), twisted gastrulation (Oelgeschläger et al., 2000), the zinc metalloproteinase Xolloid-related (Dale et al., 2002), Crossveinless-2 (Ambrosio et al., 2008; Coffinier et al., 2002; Rentzsch et al., 2006) and Sizzled (Collavin and Kirschner, 2003). Three Tolloid enzymes exist in vertebrates (Dale et al., 2002), and represent a small group of metalloproteinases, very well conserved throughout evolutionary times from Drosophila to humans (Hopkins et al., 2007). Such metalloproteases cleave Chordin at two specific sites, generating fragments that have a decreased affinity towards BMP. If that happens, the ventralizing factors, previously inactivated by Chordin, are once again enabled to promote ventralization signaling through BMP receptors (Piccolo et al., 1997). Despite being localized ventrally, Sizzled, the zebrafish homologue of which is Ogon/Mercedes (Muraoka et al., 2006), contrasts Tolloid-related proteases. It is able to inhibit the chordinase Tolloid and therefore results as an indirect feedback inhibitor of the BMP signaling in the ventral center since Chordin is found in elevated levels (De Robertis, 2009). Twisted gastrulation (Tsg) is another key factor in the play. Peculiarly, it is both a BMP- and a Chordin-binding protein (Oelgeschläger et al., 2000). On one hand, together with Chordin, it forms a ternary complex able to diffuse in the embryo's extracellular space, making it a better antagonist. On the other, because of the ventral expression site, it keeps BMP in a soluble, active state, explaining its pro-BMP effects. Then, which is the prevalent effect? Little and Mullins (2006) and Xie and Fisher (2005) showed that zebrafish Tsg-MO depletion resulted in a dorsalized phenotype, hence demonstrating Tsg's predominant pro- BMP function.

The Nodal signaling pathway is as well essential for the specification of the main body axis. It is activated within the first developmental stages, at pre-gastrula and gastrula, and is responsible for mesoderm, endoderm as well as both anterior-posterior and left–right axis formation. Nodal-related ligands of the transforming growth factor-beta (TGFbeta) superfamily exert their functions in a coordinated manner through the formation of dimers that bind to type I and II serine-threonine kinase receptors, a step that initiates a Smad-dependent signaling cascade which ultimately induces or represses transcriptional activity (Shen, 2007). In the zebrafish embryo, Sox32 – currently known as Casanova- (4.66 hpf), BON (5 hpf), Gata5 (5 hpf), Og9x – currently known as Sebox- (5 hpf), Pitx2a (5 hpf), Sox17 (5.25 hpf), Tbx16 (6 hpf) and FOXA2 (9 hpf) are few examples of factors activated by Nodal at precise developmental times (Chan et al., 2009). Goosecoid, recognized in the mid- nineties as a key factor in the formation of the body plan, is one of them. It is a dorsal-specific (Watabe et al., 1995) homeobox- containing gene (Cho et al., 1991) induced in its expression at the highest level of a molecular cascade by the Nieukoop Center and the Spemann organizer. Its detectable levels of maternal transcript at 0 and 2 hpf are very low (Stachel et al., 1993), while more prominent zygotic mRNA are available from 4 hpf (Schulte-Merker et al., 1994). Several studies have reflected its importance. When microinjected, goosecoid mRNA caused the recruitment of neighboring cells into a secondary body axis and the activation of cell movements in the injected cells (Niehrs et al., 1993). When inactivated by gene targeting to generate Goosecoid-mutant mice, craniofacial defects arose with regards to musculature including the tongue, the nasal cavity and the nasal pits, as well as the components of the inner ear, even though no gastrulation phenotypes were observed probably because of some kind of functional compensation elicited by similar genes (Rivera-Pérez et al., 1995; Yamada et al., 1995). Also, when mutated together with HNF- 3beta, a severe phenotype displaying growth defects, absence of optic vesicles, improper development of the foregut, branchial

arches and heart appeared (Filosa et al., 1997), exemplifying Goosecoid's broad interactive relationships and participation into apparently distant processes unrelated to axis patterning.

In order to disclose the outcomes of the externally-supplied beneficial bacteria on zebrafish early development, goosecoid and chordin were investigated by Miccoli et al. (2015) after supplementation with the probiotic *L. rhamnosus* to parental fish (Fig. 1H and I). Considering the higher apoptotic rates, the authors assessed whether the embryonic development of the probiotic-treated (PROBIO) group could be accelerated by the probiotic administration to their parents. First, goosecoid and chordin were reported not to show any maternal contribution. Second, they were outstandingly up regulated throughout embryonic development from 4 hpf on, when relative mRNA abundances were subjected to a five- and sixfold increase in mRNA abundance, respectively, with regards to controls. Third, such up-regulation was appreciated also by means of whole mount in situ hybridization analyses, particularly at 4 and 12 hpf, when stronger probe marks indicated a higher chordin expression at the blastoderm region and a greater distance among somites, as compared to controls, respectively (Fig. 1K).

5. miRNA processing

The concept of gene expression being regulated by RNA molecules firstly appeared in 1993 (Lee et al., 1993) and 13 years later Andrew Fire and Craig Mello won a Nobel Prize for discovering the revolutionary RNA silencing-technique called RNA interference.

Based on their biogenesis mechanism, nucleotide length, RNA silencing pathway and association with Argonaute-family proteins, small eukaryotic RNAs were grouped into three classes: microRNAs (miRNAs), endogenous small interfering RNAs (esiRNAs) and Piwi- interacting RNAs (piRNAs) (Kim et al., 2009). Yet, the ever increasing number of RNA classes discovered in eukaryotes, bacteria and archaea-currently accounting to 20 (Ghildiyal and Zamore, 2009) – makes such boundaries unclear and forces for a continuous revalidation. Small RNAs underlie a variety of functions across the genome and transcriptome, such as heterochromatin formation, mRNA destabilization and translational control (Filipowicz et al., 2008; Malone and Hannon, 2009). By targeting the very basis of life, they are involved in almost every biological process, including developmental timing, cell differentiation, cell proliferation, cell death, metabolic control, transposon silencing and antiviral defense. They hold great promise interdisciplinary-wise, with particular medical emphasis on cancer research, as highlighted by several research papers (e.g. Croce and Calin, 2005). MicroRNAs (miRNAs), in particular, are key supervisors of the post- transcriptional regulation and mRNA turnover. They are 21 nucleotide-long, single-stranded noncoding RNAs produced from double-stranded RNAs thanks to the strict, subsequent involvement of two RNase III-like enzymes, Drosha and Dicer (Muggenhumer et al., 2014).

As miRNAs serve fundamental functions, the pathway leading to their biogenesis can be subjected to regulation at several levels (Finnegan and Pasquinelli, 2013): transcriptionally by protein binding (Michlewski and Cáceres, 2010; Michlewski et al., 2011; Piskounova et al., 2011; Van Wynsberghe et al., 2011) and post- transcriptionally by ADARs (Adenosine DeAminases that act on RNA) (Hogg et al., 2011; Wulff and Nishikura, 2012) and nucleotide addition at the 30 end (Liu et al., 2011; Piskounova et al., 2011). The biogenesis pathway, quite conserved in most metazoans (Kim et al., 2009), follows discrete steps. First, the nuclear microprocessor complex composed of the RNA binding protein DGCR8 (or Pasha) and Drosha process the primary transcript, abbreviated as pri-miRNA, into pre-miRNA. Such cleavage releases a 60/70- nucleotide-long hairpin presenting a 2-nucleotide overhang at the 30 end. Then, the stem-looped pre-miRNA is exported to the cytoplasm via Exportin-5, where it is recognized and further cleaved by another 200-kDa RNase III-like enzyme, Dicer, that ultimately generate the mature 21-nt long miRNA (Bernstein et al., 2001;

Hammond et al., 2000; Hutvágner and Zamore, 2002; Hutvágner et al., 2001; Ketting et al., 2001; Knight and Bass, 2001). The resulting RNA-induced silencing complex (RISC), comprising the mature miRNA and a member of the Argonaute proteins family (recently reviewed by Hutvagner and Simard, 2008), direct either transcriptional/post-transcriptional gene silencing and/or translational repression by imperfect base pairing at the 30-UTR of target mRNAs (Wilson and Doudna, 2013).

Drosha and Dicer can be subjected to regulation (Krol et al., 2010) too, theoretically at either the transcriptional, translational, or post-translational level. Dhorne-Pollet et al. (2013), via qPCR, showed the threefold decreased amount of relative drosha mRNA abundance throughout the *Xenopus* oocyte-to-egg maturation, while protein levels increased during the same time lapse. This is due to the expression of maternally-inherited mRNAs and the extension of their poly(A) tail in the cytoplasm (Paillard and Osborne, 2003), a process on which their storage and translational activation depend. Indeed, the Drosha poly(A) tail measures 80 nt in oocytes and 200 in eggs. This demonstrates that Drosha, like every other maternally-stored mRNA, is translationally regulated by the length of its poly(A) tail (Muggenhumer et al., 2014), a practice that facilitates the translation of dormant mRNAs during oocyte development (Richter and Lasko, 2011; Villalba et al., 2011). Similarly, Dicer activity is enhanced as oocytes mature into eggs (Watanabe et al., 2005).

Moving to the *D. rerio* model, its genome assembly ‘‘Zv9” encodes for 346 miRNAs (http://www.mirbase.org/). Most of them are expressed at the segmentation stage, around 12 hpf (Rosa and Brivanlou, 2009). An exception is the miR-430 family, one of the earliest and most highly transcribed gene cluster of the zygotic genome (Chen et al., 2005; Heyn et al., 2014; Lee et al., 2013). It groups more than 70 miRNAs characterized by the 50 sequence seed AAGUGC. A single miRNA can bind up to hundreds targets, while a single mRNA can be targeted by multiple miRNAs in a combinatorial way (Rosa and Brivanlou, 2009). By their action, protein translation is silenced or mRNA deadenylation is triggered (De Moor et al., 2005; Richter, 1999), exerting a critical, yet fundamental, effect in animal development and differentiation (Bushati and Cohen, 2007).

As already hinted in the first section, a massive transcription of the zygotic genetic information is installed at the MZT. Concomitantly, clearance of maternal messages is carried out. Inherited mRNAs and proteins are indeed required for directing the early life’s processes but, as development proceeds, they become unnecessary or even possibly deleterious (Lee et al., 2014). Small RNAs at the mid-blastula transition developmental switch heavily contribute to mRNA turnover (Bushati et al., 2008; Lund et al., 2009; Marlow, 2010), an activity for which the zebrafish’s miR-430 family at the 30-UTR of selective, rather than wholesale, target genes is particularly responsible of (Giraldez et al., 2006; Mishima et al., 2006). In such period, maternal factors gradually decrease, because they are destabilized by means of 30-UTR deadenylation triggered by embryonic miRNA; in particular, miR-430 regulates morphogenesis during early development (Giraldez et al., 2006).

The erasing mechanism, in addition to the miRNAs biogenesis pathway (Kim et al., 2009), is conserved as well, as the *Xenopus* ortholog miR-427 is expressed hours before ZGA and regulates deadenylation of maternal messages (Lund et al., 2009). Noteworthy, some inherited messages are able to avoid degradation. Because of this, they keep functioning in stages where only their zygotic counterpart would normally exist, in fact cooperating with them without causing any impairment (Marlow, 2010).

As mentioned, Dicer has paramount roles. Wienholds et al. (2003), by various techniques including mutants generation, targeted gene inactivation and whole mount in situ hybridization, focused on the importance of both maternal and zygotic Dicer. They found that when homozygous and trans-heterozygous dicer1$^{-/-}$ embryos were generated, an overall arrest of growth rather than specific organogenesis defects was displayed around 8 dpf, and death of the totality of specimens was observed within three weeks post-fertilization. Likely, the initial body plan could be acquired by residual activity of the maternal Dicer: in fact, when maternal dicer was knocked-down by morpholino, embryos arrested their growth earlier.

Moreover, Giraldez et al. (2005), by means of maternal-zygotic dicer (MZdicer) mutants generation, observed that mutant embryos did not process miRNAs precursors. The machinery that would lead to maternal mRNA clearance was deactivated and, as a result, deadenylation was compromised. Despite MZdicer mutants underwent axis formation and differentiated multiple cell types, their phenotype was abnormal, as were brain formation, somitogenesis, and heart development, suggesting that clearance of maternal transcripts is essential for normal development but not for axis patterning.

Overall, authors demonstrated the importance of Dicer in zebrafish development, since embryos deficient of both maternal and zygotic Dicer were unable to generate mature miRNAs (Giraldez et al., 2006).

Herein, we report our finding regarding maternally-deposited and zygotically-transcribed dicer and drosha transcripts' abundance following parental food-associated supplementation with *L. rhamnosus*. The administration provoked severe changes in the expression patterns of both, generally causing the notable increase in the treated group's mRNA abundances (Fig. 2A and B). In particular, at 0 hpf, relative mRNA abundances were increased by approximately twofold change, hence evidencing a probiotic-driven discrete transcripts storage extent during oogenesis. At 24 hpf stage, although not statistically significant either between the two experimental groups or within the same experimental group at consecutive stages, the scenario is reversed and controls displayed slightly higher transcription levels of such miRNA processing enzymes.

6. Pluripotency maintenance signals

The transitional step of the ZGA is a major reprogramming event that induces several key processes in the embryo, such as new transcriptional activation and clearance of maternal mRNAs. Their combination results in a stage in-between of differentiated and pluripotent zygotes (Giraldez, 2010). The latter face a peculiar transcriptional regulation in a time-lapse of few hours, as maternal mRNAs are first polyadenylated in order to become functional and direct early life's development, and are then subjected to decay by means of miRNAs at their 3'-untranslated region. Across all species, both general and specific transcription factors are required for finely regulating the zygotic genome competency. The TFIID complex regulate methylation at chromatin (e.g. Vastenhouw et al., 2010), while Nanog, Pou5f1 and Sox19b intervene at the DNA level (e.g. Harvey et al., 2013; Lee et al., 2013; Potok et al., 2013). In zebrafish, the latter three are among the factors that receive polyadenylation shortly after fertilization, enabling the execution of the genetic program (Langley et al., 2014). Besides, binding sites for such pluripotency-inducing factors are extensively present on early zygotic genes (Lee et al., 2014).

Nanog, pou5f1 and sox19b are maternally supplied to the oocytes (Burgess et al., 2002; Okuda et al., 2010; Onichtchouk et al., 2010; Schuff et al., 2012; Xu et al., 2012). By RNA sequencing of 4 and 6 hpf wild-type embryos, Lee et al. (2013) found that (i) over 74% of the genes expressed at both stages have maternal contribution; (ii) zygotic genes that are directly triggered by the maternal program during the so-called ''first wave" of transcription at 4 hpf are mainly involved in axis patterning, gastrulation and chromatin remodeling (the miRNA family miR-430 is included among them); (iii) nanog, sox19b and pou5f1 are among the transcripts that are most highly translated in the pre-MZT transcriptome.

As for the second point, it is known that Nanog, Sox2, and Oct4 can participate in chromatin remodeling in embryonic stem (ES) and induced pluripotent stem (iPS) cells (Orkin and Hochedlinger, 2011). Yet, it is unclear whether their action at the nucleosome level gathers other transcription factors that would bind and act cooperatively (Zaret and Carroll, 2011). If that was

confirmed, such pluripotency-inducing proteins might play similar roles during MBT (Lee et al., 2013; Leichsenring et al., 2013), thus enabling the activation of the silent embryonic genome (Lee et al., 2014).

Among those of other transcription factors, the binding sites of Oct-3/4 (Pou5f1) gene family, Sox gene family and Nanog reside at the promoters of a set of genes involved in pluripotency and early development. The three work cooperatively to specify ES cell identity. Together with Tcf3, they bind to promoters of miRNA in ES cells (Rosa and Brivanlou, 2009) but are also known to be involved in the direct activation of the miR-430 gene cluster (Lee et al., 2013), which is one of the earliest and most highly transcribed family of the zygotic genome (Chen et al., 2005; Heyn et al., 2014; Lee et al., 2013). On the other hand, they are inhibited by miR-296, miR-470 and miR-134 at both their coding sequence and 30 UTR, to induce differentiation in mESCs (Tay et al., 2008a,b).

The first thorough indication of ESC maintenance being a multifaceted process came from Wang and colleagues (2006), who created the first pluripotency protein interaction network in mouse through whole-lane liquid chromatography–tandem mass spectrometry (LC–MS/MS). The reported map suggested several crucial findings. First, the interaction network comprises more than 80% of proteins that were demonstrated by knockout or knockdown studies to be involved in controlling the differentiation of the inner cell mass or early development, hence ensuring survival. Second, the expression of the majority of genes encoding for network's proteins is down regulated at the onset of ESC differentiation. Third, the central role of Nanog is highlighted by the fact that at least the 56% of the network's proteins coincide with Nanog and/or Oct4 putative targets found by previous ChIP analyses performed on mouse ES cell (Loh et al., 2006). Because of this, Nanog has been regarded to as a homeodomain protein. Furthermore, Nanog, Oct4 and Sox2 repress developmental genes by modulating their own expression by binding to each other's promoter regions (Saunders et al., 2013). Fourth, the network is characterized by crosslinks involved in repressing transcription by means of cofactors belonging to histone deacetylase NuRD, Polycomb and switch/sucrose non fermentable (SWI/SNF) chromatin remodeling complexes. The identification of such a large presence of physically-associated proteins acting as activators, repressors and co-repressors outlines both the multiple different regulation methods and a fail-safe mechanism for preventing undesired differentiation and maintaining staminality (Saunders et al., 2013).

Data regarding the maternal control in terms of nanog gene expression modulated by the probiotic *L. rhamnosus* are herein presented for the first time. By qPCR, we have shown that Nanog as well is heavily and positively modulated in its expression pattern by the beneficial bacteria supplemented to parent fish (Fig. 2C). At 0, 2 and 4 hpf, differences are statistically significant between groups, as the PROBIO display an overall average 2-fold increase in gene expression. As far as only the first two are considered, statistical significance exists also among consecutive developmental stages within groups.

We therefore found that the major changes in nanog mRNA storage are concentrated within the first 4 hpf. This is in accordance with Nanog's role in inducing pluripotency and mediating direct activation of the miRNA-430 family, as discussed so far.

To conclude, we tested the ability of the parentally-supplied *L. rhamnosus* to intervene in the storage of maternal information in the form of mRNAs into mature F1 oocytes, thereby demonstrating the inheritance of the physiological modification caused by the gut microbiota modulation and their effects on the autophagic, apoptotic, axes patterning, miRNA formation as well as on pluripotency maintenance processes.

The potentialities of the microbiome-gut-brain axis have been established in the last years, with remarkable consequences on health and diseases. The capability of the enteric microbiota composition, either natural or externally-manipulated, to form ecological bacterial relations that ultimately govern homeostasis is a ground-breaking subject. According to the novelty of our results

and the lack of previous reports in the scientific literature, no data existed as to the outcomes of probiotics on the maternal control, a crucial step of development. Indeed, our data have laid the foundation for understanding the broad spectrum of the possible effects concerning probiotics and animal development.

Acknowledgments

Supported by 2012–2015 COST European Cooperation in the field of Scientific and Technical Research ‘‘AQUAGAMETE” to OC, by PRIN 2010-2011 prot 2010W87LBJ to OC.

Figures

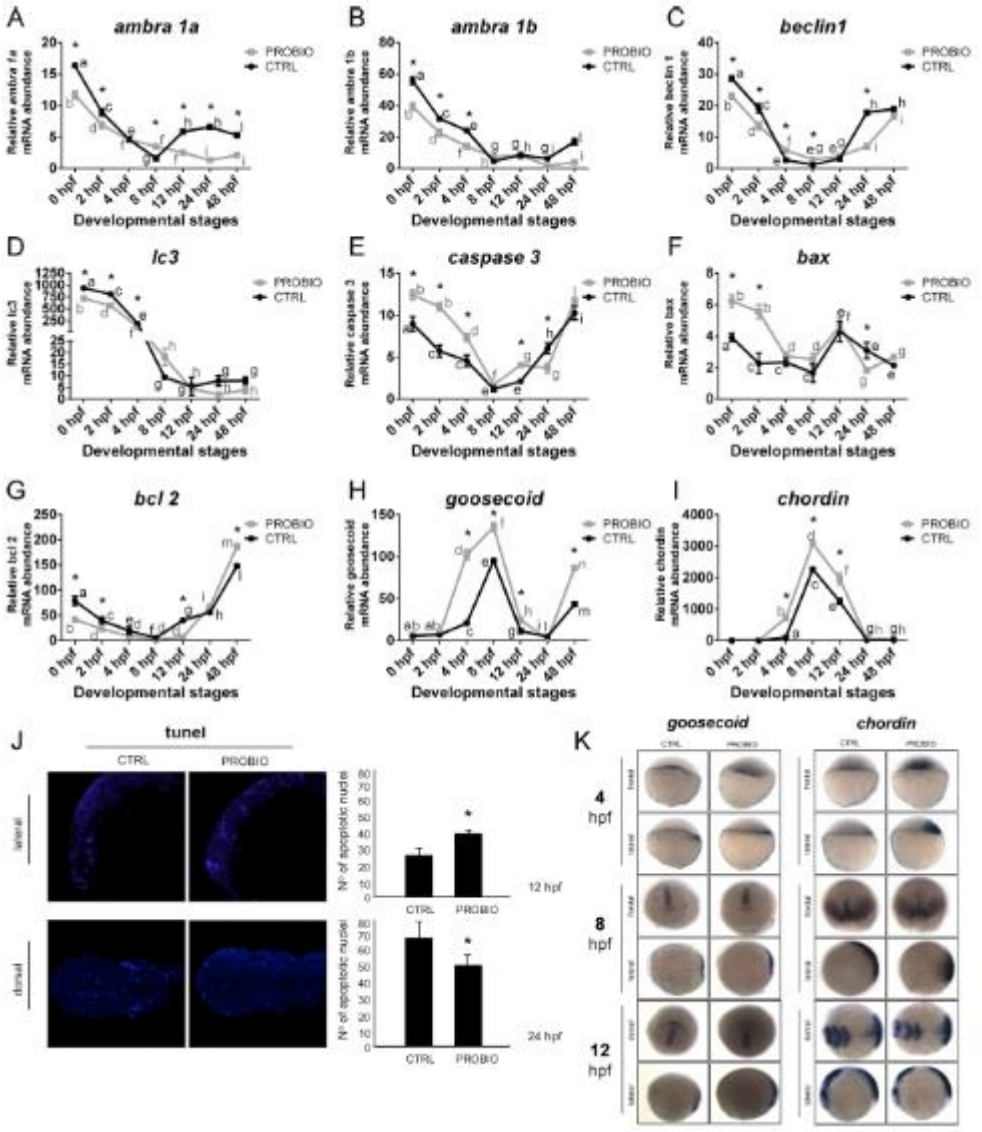

Fig.1

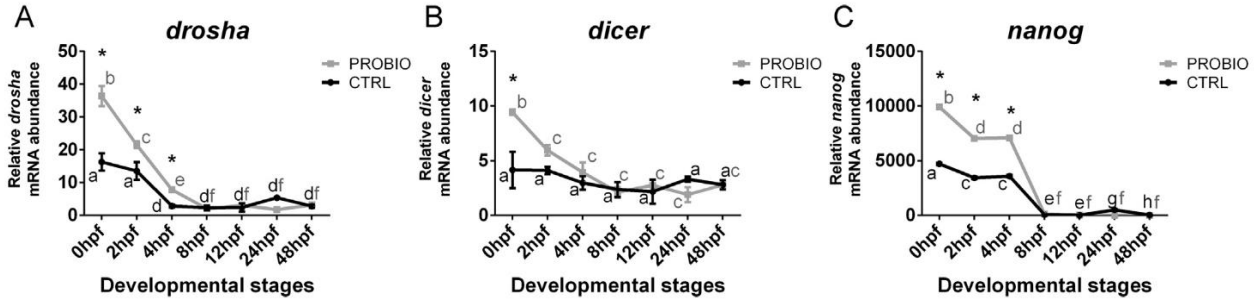

Fig. 2

Figure legends

Fig. 1. (A)–(I) Temporal expression profiles of ambra 1a1, ambra 1b, beclin 1, lc3, caspase 3, goosecoid and chordin. mRNA levels normalized against 18S for the control (CTRL) and probiotic-treated (PROBIO) groups. Error bars indicate mean ± S.D. Asterisks represent statistical difference between the two experimental groups at a given developmental stage, while letters symbolize the statistical difference within the same experimental group at consecutive developmental stages. Confidence interval set at 95% ($p < 0.05$). (J) TUNEL assay highlighting apoptotic nuclei at 12 and 24 hpf. Values are plotted as mean ± SD. Asterisk indicates significantly different number of apoptotic nuclei between experimental groups. (K) Spatial expression of goosecoid and chordin by whole mount in situ hybridization in probiotic treated and control embryos. Scale bar at goosecoid 48 hpf PROBIO: 200 µm.

Fig. 2. (A)–(C) Temporal expression profiles of drosha, dicer and nanog. Expression data normalized over against 18S. Values plotted as mean ± S.D. As for statistic details, refer to caption of Fig. 1. q-PCRs were performed with SYBR green method. All samples analyzed in triplicates. The single reaction mixture consisted of 2 µL of cDNA diluted 1:10, 10 µL of 2x concentrated iQ TM SYBR Green Supermix (Bio- Rad, 170-8882), 0.3 µM of forward and reverse primers. Forty-five cycles of amplification were run with denaturation step (30 s at 95 C) followed by the annealing stage (30 s at 60 C for all genes) and extension step for 20 s at 72 C.

References

Aanes, H., Winata, C.L., Lin, C.H., Chen, J.P., Srinivasan, K.G., Lee, S.G.P., Lim, A.Y.M., Hajan, H.S., Collas, P., Bourque, G., Gong, Z., Korzh, V., Aleström, P., Mathavan, S., 2011. Zebrafish mRNA sequencing deciphers novelties in transcriptome dynamics during maternal to zygotic transition. Genome Res. 21, 1328–1338. http://dx.doi.org/10.1101/gr.116012.110.

Aanes, H., Østrup, O., Andersen, I.S., Moen, L.F., Mathavan, S., Collas, P., Alestrom, P., 2013. Differential transcript isoform usage pre- and post-zygotic genome activation in zebrafish. BMC Genomics 14, 331. http://dx.doi.org/10.1186/1471-2164-14-331.

Abraham, M.C., Shaham, S., 2004. Death without caspases, caspases without death.

Trends Cell Biol. 14, 184–193. http://dx.doi.org/10.1016/j.tcb.2004.03.002. Akkers, R.C., van Heeringen, S.J., Jacobi, U.G., Janssen-Megens, E.M., Francoijs, K.J., Stunnenberg, H.G., Veenstra, G.J., 2009. A hierarchy of H3K4me3 and H3K27me3 acquisition in spatial gene regulation in *Xenopus* embryos. Dev. Cell 17, 425–434, doi:S1534-5807(09)00342-6, [pii] r10.1016/j. devcel.2009.08.005.

Ambrosio, A.L., Taelman, V.F., Lee, H.X., Metzinger, C.a., Coffinier, C., De Robertis, E. M., 2008. Crossveinless-2 is a BMP feedback inhibitor that binds Chordin/BMP to regulate *Xenopus* embryonic patterning. Dev. Cell 15, 248–260. http://dx.doi. org/10.1016/j.devcel.2008.06.013.

Arama, E., Agapite, J., Steller, H., 2003. Caspase activity and a specific cytochrome C are required for sperm differentiation in Drosophila. Dev. Cell 4, 687–697. http://dx.doi.org/10.1016/S1534-5807(03)00120-5.

Avella, M.a., Place, A., Du, S.J.., Williams, E., Silvi, S., Zohar, Y., Carnevali, O., 2012.

Lactobacillus rhamnosus accelerates zebrafish backbone calcification and gonadal differentiation through effects on the GnRH and IGF systems. PLoS ONE 7, 1–10. http://dx.doi.org/10.1371/journal.pone.0045572.

Babin, P., Cerdà, J., Lubzens, E., 2007. The fish oocyte: from basic studies to biotechnological applications.

Bachiller, D., Klingensmith, J., Kemp, C., Belo, J.a., Anderson, R.M., May, S.R., McMahon, J.a., McMahon, a.P., Harland, R.M., Rossant, J., De Robertis, E.M., 2000. The organizer factors Chordin and Noggin are required for mouse forebrain development. Nature 403, 658–661. http://dx.doi.org/10.1038/35001072.

Balcázar, J.L., de Blas, I., Ruiz-Zarzuela, I., Vendrell, D., Calvo, A.C., Márquez, I., Gironés, O., Muzquiz, J.L., 2007. Changes in intestinal microbiota and humoral immune response following probiotic administration in brown trout (Salmo trutta). Br. J. Nutr. 97, 522–527. http://dx.doi.org/10.1017/S0007114507432986.

Bally-Cuif, L., Schatz, W.J., Ho, R.K., 1998. Characterization of the zebrafish Orb/ CPEB-related RNA binding protein and localization of maternal components in the zebrafish oocyte. Mech. Dev. 77, 31–47.

Barski, A., Cuddapah, S., Cui, K., Roh, T.Y., Schones, D.E., Wang, Z., Wei, G., Chepelev, I., Zhao, K., 2007. High-resolution profiling of histone methylations in the human genome. Cell 129, 823–837. http://dx.doi.org/10.1016/ j.cell.2007.05.009.

Bauer, H., Meier, a., Hild, M., Stachel, S., Economides, a., Hazelett, D., Harland, R.M., Hammerschmidt, M., 1998. Follistatin and noggin are excluded from the zebrafish organizer. Dev. Biol. 204, 488–507. http://dx.doi.org/10.1006/ dbio.1998.9003.

Belmokhtar, C.a., Hillion, J., Ségal-Bendirdjian, E., 2001. Staurosporine induces apoptosis through both caspase-dependent and caspase-independent mechanisms. Oncogene 20, 3354–3362. http://dx.doi.org/10.1038/sj. onc.1204436.

Benato, F., Skobo, T., Gioacchini, G., Moro, I., Ciccosanti, F., Piacentini, M., Fimia, G.M., Carnevali, O., Valle, L.D., 2013. Ambra1 knockdown in zebrafish leads to incomplete development due to severe defects in organogenesis. Autophagy 9, 476–495. http://dx.doi.org/10.4161/auto.23278.

Bernstein, E., Caudy, a.a., Hammond, S.M., Hannon, G.J., 2001. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409, 363–366. http://dx.doi.org/10.1038/35053110.

Burgess, S., Reim, G., Chen, W., Hopkins, N., Brand, M., 2002. The zebrafish spielohne-grenzen (spg) gene encodes the POU domain protein Pou2 related to mammalian Oct4 and is essential for formation of the midbrain and hindbrain, and for pre-gastrula morphogenesis. Development 129, 905–916.

Bushati, N., Cohen, S.M., 2007. MicroRNA functions. Annu. Rev. Cell Dev. Biol. 23, 175–205. http://dx.doi.org/10.1146/annurev.cellbio.23.090506.123406.

Bushati, N., Stark, A., Brennecke, J., Cohen, S.M., 2008. Temporal reciprocity of miRNAs and their targets during the maternal-to-zygotic transition in Drosophila. Curr. Biol. 18, 501–506. http://dx.doi.org/10.1016/j.cub.2008.02.081.

Carnevali, O., de Vivo, L., Sulpizio, R., Gioacchini, G., Olivotto, I., Silvi, S., Cresci, A., 2006. Growth improvement by probiotic in European sea bass juveniles (Dicentrarchus labrax L.), with particular attention to IGF-1, myostatin and cortisol gene expression. Aquaculture 258, 430–438. http://dx.doi.org/10.1016/ j.aquaculture.2006.04.025.

Carnevali, O., Avella, M.A., Gioacchini, G., 2013. Effects of probiotic administration on zebrafish development and reproduction. Gen. Comp. Endocrinol. 188, 297–302.

Castex, M., Chim, L., Pham, D., Lemaire, P., Wabete, N., Nicolas, J.L., Schmidely, P., Mariojouls, C., 2008. Probiotic P. acidilactici application in shrimp Litopenaeus stylirostris culture subject to vibriosis in New Caledonia. Aquaculture 275, 182–193. http://dx.doi.org/10.1016/j.aquaculture.2008.01.011.

Cecconi, F., Piacentini, M., Fimia, G.M., 2008. The involvement of cell death and survival in neural tube defects: a distinct role for apoptosis and autophagy? Cell Death Differ. 15, 1170–1177. http://dx.doi.org/10.1038/cdd.2008.64.

Chan, T.M., Longabaugh, W., Bolouri, H., Chen, H.L., Tseng, W.F., Chao, C.H., Jang, T.H., Lin, Y.I., Hung, S.C., Wang, H.D., Yuh, C.H., 2009. Developmental gene regulatory networks in the zebrafish embryo. Biochim. Biophys. Acta 1789, 279–298. http://dx.doi.org/10.1016/j.bbagrm.2008.09.005.

Chen, P.Y., Manninga, H., Slanchev, K., Chien, M., Russo, J.J., Ju, J., Sheridan, R., John, B., Marks, D.S., Gaidatzis, D., Sander, C., Zavolan, M., Tuschl, T., 2005. The developmental miRNA profiles of zebrafish as determined by small RNA cloning. Genes Dev. 19, 1288–1293. http://dx.doi.org/10.1101/gad.1310605.

Chen, K., Johnston, J., Shao, W., Meier, S., Staber, C., Zeitlinger, J., 2013. A global change in RNA polymerase II pausing during the Drosophila midblastula transition. Elife 2013, 1–19. http://dx.doi.org/10.7554/eLife.00861.

Cho, K.W., Blumberg, B., Steinbeisser, H., De Robertis, E.M., 1991. Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene goosecoid. Cell 67, 1111–1120. http://dx.doi.org/10.1016/0092-8674(91)90288-A.

Chowdhury, I., Tharakan, B., Bhat, G.K., 2008. Caspases – an update. Comp. Biochem.Physiol. – B Biochem. Mol. Biol. 151, 10–27. http://dx.doi.org/10.1016/j. cbpb.2008.05.010.

Chun, H.J., Zheng, L., Ahmad, M., Wang, J., Speirs, C.K., Siegel, R.M., Dale, J.K., Puck, J., Davis, J., Hall, C.G., Skoda-Smith, S., Atkinson, T.P., Straus, S.E., Lenardo, M.J., 2002. Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. Nature 419, 395–399. http://dx. doi.org/10.1038/nature01063.

Cianfanelli, V., De Zio, D., Di Bartolomeo, S., Nazio, F., Strappazzon, F., Cecconi, F.,2015. Ambra1 at a glance. J. Cell Sci. 128, 2003–2008. http://dx.doi.org/10.1242/jcs.168153.

Ciechomska, I.a., Goemans, G.C., Skepper, J.N., Tolkovsky, a.M., 2009. Bcl-2 complexed with Beclin-1 maintains full anti-apoptotic function. Oncogene 28, 2128–2141. http://dx.doi.org/10.1038/onc.2009.60.

Coffinier, C., Ketpura, N., Tran, U., Geissert, D., De Robertis, E.M., 2002. Mouse crossveinless-2 is the vertebrate homolog of a Drosophila extracellular regulator of BMP signaling. Gene Expr. Patterns 2, 189–194. http://dx.doi.org/10.1016/S0925-4773(02)00420-3.

Cole, L.K., Ross, L.S., 2001. Apoptosis in the developing zebrafish embryo. Dev. Biol. 240, 123–142. http://dx.doi.org/10.1006/dbio.2001.0432.

Collart, C., Owens, N.D.L., Bhaw-Rosun, L., Cooper, B., De Domenico, E., Patrushev, I., Sesay, A.K., Smith, J.N., Smith, J.C., Gilchrist, M.J., 2014. High-resolution analysis of gene activity during the *Xenopus* mid-blastula transition. Development 141, 1927–1939. http://dx.doi.org/10.1242/dev.102012.

Collavin, L., Kirschner, M.W., 2003. The secreted Frizzled-related protein Sizzled functions as a negative feedback regulator of extreme ventral mesoderm. Development 130, 805–816. http://dx.doi.org/10.1242/dev.00306.

Cory, S., Adams, J.M., 2002. The Bcl2 family: regulators of the cellular life-or-death switch. Nat. Rev. Cancer 2, 647–656. http://dx.doi.org/10.1038/nrc883.

Croce, C.M., Calin, G.A., 2005. MiRNAs, cancer, and stem cell division. Cell 122, 6–7. http://dx.doi.org/10.1016/j.cell.2005.06.036.

Cryan, J.F., O'Mahony, S.M., 2011. The microbiome-gut-brain axis: from bowel to behavior. Neurogastroenterol. Motil. 23, 187–192. http://dx.doi.org/10.1111/ j.1365-2982.2010.01664.x.

Dae, Y.K., Roy, R., 2006. Cell cycle regulators control centrosome elimination during oogenesis in Caenorhabditis elegans. J. Cell Biol. 174, 751–757. http://dx.doi.org/10.1083/jcb.200512160.

Dale, L., Evans, W., Goodman, S.A., 2002. Xolloid-related: a novel BMP1/Tolloid- related metalloprotease is expressed during early *Xenopus* development. Mech. Dev. 119, 177–190. http://dx.doi.org/10.1016/S0925-4773(02)00359-3.

Dal-Pra, S., Fürthauer, M., Van-Celst, J., Thisse, B., Thisse, C., 2006. Noggin1 and Follistatin-like2 function redundantly to Chordin to antagonize BMP activity. Dev. Biol. 298, 514–526. http://dx.doi.org/10.1016/j.ydbio.2006.07.002.

De Moor, C.H., Meijer, H., Lissenden, S., 2005. Mechanisms of translational control by the 30 UTR in development and differentiation. Semin. Cell Dev. Biol. 16, 49–58. http://dx.doi.org/10.1016/j.semcdb.2004.11.007.

De Renzis, S., Elemento, O., Tavazoie, S., Wieschaus, E.F., 2007. Unmasking activation of the zygotic genome using chromosomal deletions in the Drosophila embryo. PLoS Biol. 5, 1036–1051. http://dx.doi.org/10.1371/journal.pbio.0050117.

De Robertis, E.M., 2009. Spemann's organizer and the self-regulation of embryonic fields. Mech. Dev. 126, 925–941. http://dx.doi.org/10.1016/j.mod.2009.08.004.

Deter, R.L., De Duve, C., 1967. Influence of glucagon, an inducer of cellular autophagy, on some physical properties of rat liver lysosomes. J. Cell Biol. 33, 437–449. http://dx.doi.org/10.1083/jcb.33.2.437.

Dhorne-Pollet, S., Thelie, A., Pollet, N., 2013. Validation of novel reference genes for RT-qPCR studies of gene expression in *Xenopus* tropicalis during embryonic and post-embryonic development. Dev. Dyn. 242, 709–717. http://dx.doi.org/10.1002/dvdy.23972.

Di Bartolomeo, S., Corazzari, M., Nazio, F., Oliverio, S., Lisi, G., Antonioli, M., Pagliarini, V., Matteoni, S., Fuoco, C., Giunta, L., D'Amelio, M., Nardacci, R., Romagnoli, A., Piacentini, M., Cecconi, F., Fimia, G.M., 2010. The dynamic interaction of AMBRA1 with the dynein motor complex regulates mammalian autophagy. J. Cell Biol. 191, 155–168. http://dx.doi.org/10.1083/jcb.201002100.

Dunican, D.S., Ruzov, A., Hackett, J.a., Meehan, R.R., 2008. XDnmt1 regulates transcriptional silencing in pre-MBT *Xenopus* embryos independently of its catalytic function. Development 135, 1295–1302. http://dx.doi.org/10.1242/dev.016402.

Ellis, R.E., Yuan, J.Y., Horvitz, H.R., 1991. Mechanisms and functions of cell death. Annu. Rev. Cell Biol. 7, 663–698. http://dx.doi.org/10.1146/annurev.cellbio.7.1.663.

Elmore, S., 2007. Apoptosis: a review of programmed cell death. Toxicol. Pathol. 35, 495–516. http://dx.doi.org/10.1080/01926230701320337.

Endo, K., Mizuguchi, M., Harata, A., Itoh, G., Tanaka, K., 2010. Nocodazole induces mitotic cell death with apoptotic-like features in Saccharomyces cerevisiae. FEBS Lett. 584, 2387–2392. http://dx.doi.org/10.1016/j.febslet.2010.04.029.

Erlich, S., Mizrachy, L., Segev, O., Lindenboim, L., Zmira, O., Adi-Harel, S., Hirsch, J.A., Stein, R., Pinkas-Kramarski, R., 2007. Differential interactions between Beclin 1 and Bcl-2 family members. Autophagy 3, 561–568.

Fainsod, A., Steinbeisser, H., De Robertis, E.M., 1994. On the function of BMP-4 in patterning the marginal zone of the *Xenopus* embryo. EMBO J. 13, 5015–5025.

Falcinelli, S., Picchietti, S., Rodiles, A., Cossignani, L., Merrifield, D.L., Taddei, A.R., Maradonna, F., Olivotto, I., Gioacchini, G., Carnevali, O., 2015. *Lactobacillus rhamnosus* lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism. Sci. Rep. 5, 9336. http://dx. doi.org/10.1038/srep09336.

Falcinelli, S., Rodiles, A., Unniappan, S., Picchietti, S., Gioacchini, G., Merrifield, D.L., Carnevali, O., 2016. Probiotic treatment reduces appetite and glucose level in the zebrafish model. Sci. Rep. 6, 18061. http://dx.doi.org/10.1038/srep18061.

Feng, X.-H., Derynck, R., 2005. Specificity and versatility in tgf-beta signaling through Smads. Annu. Rev. Cell Dev. Biol. 21, 659–693. http://dx.doi.org/10.1146/annurev.cellbio.21.022404.142018.

Filipowicz, W., Bhattacharyya, S.N., Sonenberg, N., 2008. Mechanisms of post- transcriptional regulation by microRNAs: are the answers in sight? Nat. Rev. Genet. 9, 102–114. http://dx.doi.org/10.1038/nrg2290.

Filosa, S., Rivera-Pérez, J.a., Gómez, a.P., Gansmuller, a., Sasaki, H., Behringer, R.R., Ang, S.L., 1997. Goosecoid and HNF-3beta genetically interact to regulate neural tube patterning during mouse embryogenesis. Development 124, 2843–2854.

Fimia, G.M., Stoykova, A., Romagnoli, A., Giunta, L., Di Bartolomeo, S., Nardacci, R., Corazzari, M., Fuoco, C., Ucar, A., Schwartz, P., Gruss, P., Piacentini, M., Chowdhury, K., Cecconi, F., 2007. Ambra1 regulates autophagy and development of the nervous system. Nature 447, 1121–1125. http://dx.doi. org/10.1038/nature05925.

Finnegan, E.F., Pasquinelli, A.E., 2013. MicroRNA biogenesis: regulating the regulators. Crit. Rev. Biochem. Mol. Biol. 48, 51–68. http://dx.doi.org/10.3109/10409238.2012.738643.

Funderburk, S.F., Wang, Q.J., Yue, Z., 2010. The Beclin 1-VPS34 complex – at the crossroads of autophagy and beyond. Trends Cell Biol. 20, 355–362. http://dx. doi.org/10.1016/j.tcb.2010.03.002.

Furuya, N., Yu, J., Byfield, M., Pattingre, S., Levine, B., 2014. The evolutionarily conserved domain of Beclin 1 is required for Vps34 binding, autophagy, and tumor suppressor function. Autophagy 1, 46–52. http://dx.doi.org/10.4161/ auto.1.1.1542.

Gasparini, F., Skobo, T., Benato, F., Gioacchini, G., Voskoboynik, A., Carnevali, O., Manni, L., Dalla Valle, L., 2016. Characterization of Ambra1 in asexual cycle of a non-vertebrate chordate, the colonial tunicate Botryllus schlosseri, and phylogenetic analysis of the protein group in Bilateria. Mol. Phylogenet. Evol.95, 46–57. http://dx.doi.org/10.1016/j.ympev.2015.11.001.

Ghildiyal, M., Zamore, P.D., 2009. Small silencing RNAs: an expanding universe. Nat.Rev. Genet. 10, 94–108. http://dx.doi.org/10.1038/nrg2504.

Gioacchini, G., Giorgini, E., Merrifield, D.L., Hardiman, G., Borini, A., Vaccari, L., Carnevali, O., 2012. Probiotics can induce follicle maturational competence: the *Danio rerio* case. Biol. Reprod. 86, 65. http://dx.doi.org/10.1095/ biolreprod.111.094243.

Gioacchini, G., Dalla Valle, L., Benato, F., Fimia, G.M., Nardacci, R., Ciccosanti, F., Piacentini, M., Borini, A., Carnevali, O., 2013. Interplay between autophagy and apoptosis in the development of *Danio rerio* follicles and the effects of a probiotic. Reprod. Fertil. Dev. 25, 1115–1125. http://dx.doi.org/10.1071/ RD12187.

Gioacchini, G., Giorgini, E., Olivotto, I., Maradonna, F., Merrifield, D.L., Carnevali, O., 2014. The influence of probiotics on zebrafish *Danio rerio* innate immunity and hepatic stress. Zebrafish 11, 98–106. http://dx.doi.org/10.1089/zeb.2013.0932.

Giraldez, A.J., 2010. MicroRNAs, the cell's Nepenthe: clearing the past during the maternal-to-zygotic transition and cellular reprogramming. Curr. Opin. Genet. Dev. 20, 369–375. http://dx.doi.org/10.1016/j.gde.2010.04.003.

Giraldez, A.J., Cinalli, R.M., Glasner, M.E., Enright, A.J., Thomson, J.M., Baskerville, S., Hammond, S.M., Bartel, D.P., Schier, A.F., 2005. MicroRNAs regulate brain morphogenesis in zebrafish. Science 308, 833–838. http://dx.doi.org/10.1126/science.1109020.

Giraldez, A.J., Mishima, Y., Rihel, J., Grocock, R.J., Van Dongen, S., Inoue, K., Enright, A.J., Schier, A.F., 2006. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. Science 312 (80), 75–79. http://dx.doi.org/10.1126/science.1122689.

Glick, D., Barth, S., Macleod, K.F., 2010. Autophagy: cellular and molecular mechanisms. J. Pathol. 221, 3–12. http://dx.doi.org/10.1002/path.2697. Autophagy.

Greenwood, J., Gautier, J., 2005. From oogenesis through gastrulation: developmental regulation of apoptosis. Semin. Cell Dev. Biol. 16, 215–224. http://dx.doi.org/10.1016/j.semcdb.2004.12.002.

Grinblat, Y., Gamse, J., Patel, M., Sive, H., 1998. Determination of the zebrafish forebrain: induction and patterning. Development 125, 4403–4416.

Groisman, I., Jung, M.-Y., Sarkissian, M., Cao, Q., Richter, J.D., 2002. Translational control of the embryonic cell cycle. Cell 109, 473–483. http://dx.doi.org/10.1016/S0092-8674(02)00733-X.

Haberle, V., Li, N., Hadzhiev, Y., Plessy, C., Previti, C., Nepal, C., Gehrig, J., Dong, X., Akalin, A., Suzuki, A.M., van IJcken, W.F.J., Armant, O., Ferg, M., Strähle, U., Carninci, P., Müller, F., Lenhard, B., 2014. Two independent transcription initiation codes overlap on vertebrate core promoters. Nature 507, 381–385. http://dx.doi.org/10.1038/nature12974.

Hakem, R., Hakem, A., Duncan, G.S., Henderson, J.T., Woo, M., Soengas, M.S., Elia, A., De La Pompa, J.L., Kagi, D., Khoo, W., Potter, J., Yoshida, R., Kaufman, S.a, Lowe, S. W., Penninger, J.M., Mak, T.W., 1998. Differential requirement for Caspase 9 in apoptotic pathways in vivo. Cell 94, 339–352. http://dx.doi.org/10.1016/S0092-8674(00)81477-4.

Hamatani, T., Hamatani, T., Carter, M.G., Carter, M.G., Sharov, A.a., Ko, M.S.H., Ko, M.S.H., 2004. Dynamics of global gene expression changes during mouse preimplantation development. Dev. Cell 6, 117–131. http://dx.doi.org/10.1016/S1534-5807(03)00373-3.

Hammerschmidt, M., Pelegri, F., Mullins, M.C., Kane, D.A., van Eeden, F.J., Granato, M., Brand, M., Furutani-Seiki, M., Haffter, P., Heisenberg, C.P., Jiang, Y.J., Kelsh, R. N., Odenthal, J., Warga, R.M., Nusslein-Volhard, C., 1996. Dino and mercedes, two genes regulating dorsal development in the zebrafish embryo. Development 123, 95–102.

Hammond, S.M., Bernstein, E., Beach, D., Hannon, G.J., 2000. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 404, 293–296. http://dx.doi.org/10.1038/35005107.

Harvey, S.a, Sealy, I., Kettleborough, R., Fenyes, F., White, R., Stemple, D., Smith, J.C., 2013. Identification of the zebrafish maternal and paternal transcriptomes. Development 140, 2703–2710. http://dx.doi.org/10.1242/dev.095091.

He, C., Bartholomew, C.R., Zhou, W., Klionsky, D.J., 2009. Assaying autophagic activity in transgenic GFP-Lc3 and GFP-Gabarap zebrafish embryos. Autophagy 5, 520–526. http://dx.doi.org/10.4161/auto.5.4.7768.

Heasman, J., 1997. Patterning the *Xenopus* blastula. Development 124, 4179–4191. Hensey, C., Gautier, J., 1997. A developmental timer that regulates apoptosis at the onset of gastrulation. Mech. Dev. 69, 183–195. http://dx.doi.org/10.1016/S0925-4773(97)00191-3.

Heyn, P., Kircher, M., Dahl, A., Kelso, J., Tomancak, P., Kalinka, A.T., Neugebauer, K.M., 2014. The earliest transcribed zygotic genes are short, newly evolved, and different across species. Cell Rep. 6, 285–292. http://dx.doi.org/10.1016/j. celrep.2013.12.030.

Hogg, M., Paro, S., Keegan, L.P., O'Connell, M.a., 2011. RNA editing by mammalian ADARs. Adv. Genet. http://dx.doi.org/10.1016/B978-0-12-380860-8.00003-3. Hopkins, D.R., Keles, S., Greenspan, D.S., 2007. The bone morphogenetic protein 1/Tolloid-like metalloproteinases. Matrix Biol. 26, 508–523. http://dx.doi.org/10.1016/j.matbio.2007.05.004.

Howe, J.a., Howell, M., Hunt, T., Newport, J.W., 1995. Identification of a developmental timer regulating the stability of embryonic cyclin A and a new somatic A-type cyclin at gastrulation. Genes Dev. 9, 1164–1176. http://dx.doi. org/10.1101/gad.9.10.1164.

Howley, C., Ho, R.K., 2000. MRNA localization patterns in zebrafish oocytes. Mech.Dev. 92, 305–309. http://dx.doi.org/10.1016/S0925-4773(00)00247-1. Hutvagner, G., Simard, M.J., 2008. Argonaute proteins: key players in RNA silencing. Nat. Rev. Mol. Cell Biol. 9, 22–32. http://dx.doi.org/10.1038/nrm2321.

Hutvágner, G., Zamore, P.D., 2002. A microRNA in a multiple-turnover RNAi enzyme complex. Science 297, 2056–2060. http://dx.doi.org/10.1126/science.1073827. Hutvágner, G., McLachlan, J., Pasquinelli, A.E., Bálint, E., Tuschl, T., Zamore, P.D., 2001. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 293, 834–838. http://dx. doi.org/10.1126/science.1062961.

Igney, F.H., Krammer, P.H., 2002. Death and anti-death: tumour resistance to apoptosis. Nat. Rev. Cancer 2, 277–288. http://dx.doi.org/10.1038/nrc776.

Ikegami, R., Zhang, J., Rivera-Bennetts, a K., Yager, T.D., 1997. Activation of the metaphase checkpoint and an apoptosis programme in the early zebrafish embryo, by treatment with the spindle-destabilising agent nocodazole. Zygote 5, 329–350. http://dx.doi.org/10.1017/S0967199400003919.

Ikegami, R., Hunter, P., Yager, T.D., 1999. Developmental activation of the capability to undergo checkpoint-induced apoptosis in the early zebrafish embryo. Dev. Biol. 209, 409–433. http://dx.doi.org/10.1006/dbio.1999.9243.

Imai, Y., Gates, M.a, Melby, a.E., Kimelman, D., Schier, a.F., Talbot, W.S., 2001. The homeobox genes vox and vent are redundant repressors of dorsal fates in zebrafish. Development 128, 2407–2420.

Inomata, H., Haraguchi, T., Sasai, Y., 2008. Robust stability of the embryonic axial pattern requires a secreted scaffold for chordin degradation. Cell 134, 854–865. http://dx.doi.org/10.1016/j.cell.2008.07.008.

Jette, C.a., Flanagan, a.M., Ryan, J., Pyati, U.J., Carbonneau, S., Stewart, R.a., Langenau, D.M., Look, a.T., Letai, a., 2008. BIM and other BCL-2 family proteins exhibit cross-species conservation of function between zebrafish and mammals. Cell Death Differ. 15, 1063–1072. http://dx.doi.org/10.1038/cdd.2008.42.

Jiang, L., Zhang, J., Wang, J.J., Wang, L., Zhang, L., Li, G., Yang, X., Ma, X., Sun, X., Cai, J., Zhang, J., Huang, X., Yu, M., Wang, X., Liu, F., Wu, C.I., He, C., Zhang, B., Ci, W., Liu, J., 2013. Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. Cell 153, 773–784. http://dx.doi.org/10.1016/j.cell.2013.04.041.

Kane, D.A., Kimmel, C.B., 1993. The zebrafish midblastula transition. Development 119, 447–456.

Kang, T.-B., Ben-Moshe, T., Varfolomeev, E.E., Pewzner-Jung, Y., Yogev, N., Jurewicz, A., Waisman, A., Brenner, O., Haffner, R., Gustafsson, E., Ramakrishnan, P., Lapidot, T., Wallach, D., 2004. Caspase-8 serves both apoptotic and nonapoptotic roles. J. Immunol. 173, 2976–2984. http://dx.doi.org/10.4049/ jimmunol.173.5.2976.

Kemphues, K.J., Strome, S., 1997. Fertilization and establishment of polarity in the embryo. In: Riddle, D.L., Blumenthal, T., Meyer, B.J., Priess, J.R. (Eds.), C. Elegans II. Cold Spring Harbor Laboratory Press.

Kerr, J.F., Wyllie, A.H., Currie, A.R., 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26, 239–257. http://dx.doi.org/10.1111/j.1365-2796.2005.01570.x.

Ketting, R.F., Fischer, S.E., Bernstein, E., Sijen, T., Hannon, G.J., Plasterk, R.H., 2001. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes Dev. 15, 2654–2659. http://dx.doi. org/10.1101/gad.927801.

Khokha, M.K., Yeh, J., Grammer, T.C., Harland, R.M., 2005. Depletion of three BMP antagonists from Spemann's organizer leads to a catastrophic loss of dorsal structures. Dev. Cell 8, 401–411. http://dx.doi.org/10.1016/j. devcel.2005.01.013.

Kihara, A., Kabeya, Y., Ohsumi, Y., Yoshimori, T., 2001. Beclin-phosphatidylinositol 3-kinase complex functions at the trans-Golgi network. EMBO Rep. 2, 330–335. http://dx.doi.org/10.1093/embo-reports/kve061.

Kim, V.N., Han, J., Siomi, M.C., 2009. Biogenesis of small RNAs in animals. Nat. Rev. Mol. Cell Biol. 10, 126–139. http://dx.doi.org/10.1038/nrm2632.

Klionsky, D.J., Emr, S.D., 2000. Autophagy as a regulated pathway of cellular degradation. Science 290, 1717–1721. http://dx.doi.org/10.1126/science.290.5497.1717.

Klionsky, D.J., Cregg, J.M., Dunn, W.A., Emr, S.D., Sakai, Y., Sandoval, I.V., Sibirny, A., Subramani, S., Thumm, M., Veenhuis, M., Ohsumi, Y., 2003. A unified nomenclature for yeast autophagy-related genes. Dev. Cell 5, 539–545. http:// dx.doi.org/10.1016/S1534-5807(03)00296-X.

Klionsky, D.J., Eskelinen, E.-L., Deretic, V., 2014. Autophagosomes, phagosomes, autolysosomes, phagolysosomes, autophagolysosomes.. . wait, I'm confused. Autophagy 10, 549–551. http://dx.doi.org/10.4161/auto.28448.

Knight, S.W., Bass, B.L., 2001. A role for the RNase III DCR-1 in RNA interference and germ line development in C. elegans. Science 293 (80), 2269–2271.

Koos, D.S., Ho, R.K., 1999. The nieuwkoid/dharma homeobox gene is essential for bmp2b repression in the zebrafish pregastrula. Dev. Biol. 215, 190–207. http:// dx.doi.org/10.1006/dbio.1999.9479.

Koshida, S., Shinya, M., Mizuno, T., Kuroiwa, a., Takeda, H., 1998. Initial anteroposterior pattern of the zebrafish central nervous system is determined by differential competence of the epiblast. Development 125, 1957–1966.

Kratz, E., Eimon, P.M., Mukhyala, K., Stern, H., Zha, J., Strasser, a., Hart, R., Ashkenazi, a., 2006. Functional characterization of the Bcl-2 gene family in the zebrafish. Cell Death Differ. 13, 1631–1640. http://dx.doi.org/10.1038/sj.cdd.4402016.

Krol, J., Loedige, I., Filipowicz, W., 2010. The widespread regulation of microRNA biogenesis, function and decay. Nat. Rev. Genet. 11, 597–610. http://dx.doi.org/10.1038/nrg2843.

Kuida, K., Zheng, T.S., Na, S., Kuan, C.-Y., Yang, D., Karasuyama, H., Rakic, P., Flavell, R.A., 1996. Decreased apoptosis in the brain and premature lethality in CPP32- deficient mice. Nature 384, 368–372. http://dx.doi.org/10.1038/384368a0.

Langley, A.R., Smith, J.C., Stemple, D.L., Harvey, S.A., 2014. New insights into the maternal to zygotic transition. Development 141, 3834–3841. http://dx.doi.org/10.1242/dev.102368.

Le Good, J.A., Joubin, K., Giraldez, A.J., Ben-Haim, N., Beck, S., Chen, Y., Schier, A.F., Constam, D.B., 2005. Nodal stability determines signaling range. Curr. Biol. 15, 31–36. http://dx.doi.org/10.1016/j.cub.2004.12.062.

Lee, R.C., Feinbaum, R.L., Ambros, V., 1993. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75, 843–854. http://dx.doi.org/10.1016/0092-8674(93)90529-Y.

Lee, M.T., Bonneau, A.R., Takacs, C.M., Bazzini, A.a., DiVito, K.R., Fleming, E.S., Giraldez, A.J., 2013. Nanog, Pou5f1 and SoxB1 activate zygotic gene expression during the maternal-to-zygotic transition. Nature 503, 360–364. http://dx.doi. org/10.1038/nature12632.

Lee, M.T., Bonneau, A.R., Giraldez, A.J., 2014. Zygotic genome activation during the maternal-to-zygotic transition. Annu. Rev. Cell Dev. Biol. 30, 581–613. http://dx. doi.org/10.1146/annurev-cellbio-100913-013027.

Leichsenring, M., Maes, J., Mössner, R., Driever, W., Onichtchouk, D., 2013. Pou5f1 transcription factor controls zygotic gene activation in vertebrates. Science (New York, NY) 341, 1005–1009. http://dx.doi.org/10.1126/science.1242527.

Levine, B., Klionsky, D.J., 2004. Development by self-digestion molecular mechanisms and biological functions of autophagy. Dev. Cell 6, 463–477. http://dx.doi.org/10.1016/S1534-5807(04)00099-1.

Li, Z., Chen, B., Wu, Y., Jin, F., Liu, X., Xia, Y., 2010. Genetic and epigenetic silencing of the Beclin 1 gene in sporadic breast tumors. BMC Cancer 10, 98. http://dx.doi. org/10.1186/1471-2407-10-98.

Liang, X.H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., Levine, B., 1999. Induction of autophagy and inhibition of tumorigenesis by Beclin 1. Nature 402, 672–676. http://dx.doi.org/10.1038/45257.

Lindeman, L.C., Andersen, I.S., Reiner, A.H., Li, N., Aanes, H., Østrup, O., Winata, C., Mathavan, S., Müller, F., Aleström, P., Collas, P., 2011. Prepatterning of developmental gene expression by modified histones before zygotic genome activation. Dev. Cell 21, 993–1004. http://dx.doi.org/10.1016/j. devcel.2011.10.008.

Little, S.C., Mullins, M.C., 2006. Extracellular modulation of BMP activity in patterning the dorsoventral axis. Birth Defects Res. C Embryo Today 78, 224–242. http://dx.doi.org/10.1002/bdrc.20079.

Liu, N., Abe, M., Sabin, L.R., Hendriks, G.J., Naqvi, A.S., Yu, Z., Cherry, S., Bonini, N.M., 2011. The exoribonuclease nibbler controls 30 end processing of microRNAs in drosophila. Curr. Biol. 21, 1888–1893. http://dx.doi.org/10.1016/j. cub.2011.10.006.

Loh, Y.-H., Wu, Q., Chew, J.-L., Vega, V.B., Zhang, W., Chen, X., Bourque, G., George, J., Leong, B., Liu, J., Wong, K.-Y., Sung, K.W., Lee, C.W.H., Zhao, X.-D., Chiu, K.-P., Lipovich, L., Kuznetsov, V.a., Robson, P., Stanton, L.W., Wei, C.-L., Ruan, Y., Lim, B., Ng, H.-H., 2006. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. Nat. Genet. 38, 431–440. http:// dx.doi.org/10.1038/ng1760.

Lund, E., Liu, M., Hartley, R.S., Sheets, M.D., Dahlberg, J.E., 2009. Deadenylation of maternal mRNAs mediated by miR-427 in *Xenopus* laevis embryos. RNA 15, 2351–2363. http://dx.doi.org/10.1261/rna.1882009.

Luo, S., Rubinsztein, D.C., 2009. Apoptosis blocks Beclin 1-dependent autophagosome synthesis: an effect rescued by Bcl-xL. Cell Death Differ. 17, 268–277. http://dx.doi.org/10.1038/cdd.2009.121.

Maiuri, M.C., Criollo, A., Tasdemir, E., Vicencio, J.M., Tajeddine, N., Hickman, J.a, Geneste, O, Kroemer, G, 2007a. BH3-only proteins and BH3 mimetics induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-XL. Autophagy 3, 374–376, 4237 [pii].

Maiuri, M.C., Zalckvar, E., Kimchi, A., Kroemer, G., 2007b. Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat. Rev. Mol. Cell Biol. 8, 741–752. http://dx.doi.org/10.1038/nrm2239.

Malone, C.D., Hannon, G.J., 2009. Small RNAs as guardians of the genome. Cell 136, 656–668. http://dx.doi.org/10.1016/j.cell.2009.01.045.

Maradonna, F., Gioacchini, G., Falcinelli, S., Bertotto, D., Radaelli, G., Olivotto, I., Carnevali, O., 2013. Probiotic supplementation promotes calcification in *Danio rerio* larvae: a molecular study. PLoS ONE 8, e83155. http://dx.doi.org/10.1371/ journal.pone.0083155.

Marlow, F.L., 2010. Maternal control of development in vertebrates. Colloquium Ser. Dev. Biol. http://dx.doi.org/10.4199/C00023ED1V01Y201012DEB005.

Marzella, L., Ahlberg, J., Glaumann, H., 1981. Autophagy, heterophagy, microautophagy and crinophagy as the means for intracellular degradation. Virchows Arch. B. Cell Pathol. Incl. Mol. Pathol. 36, 219–234. http://dx.doi.org/10.1007/BF02912068.

Masaki, R., Yamamoto, A., Tashiro, Y., 1987. Cytochrome P-450 and NADPH- cytochrome P-450 reductase are degraded in the autolysosomes in rat liver. J. Cell Biol. 104, 1207–1215.

Meier, P., Finch, A., Evan, G., 2000. Apoptosis in development. Nature 407, 796–801. http://dx.doi.org/10.1038/35037734.

Mendez, R., Richter, J.D., 2001. Translational control by CPEB: a means to the end. Nat. Rev. Mol. Cell Biol. 2, 521–529. http://dx.doi.org/10.1038/35080081. Metzstein, M.M., Stanfield, G.M., Horvitz, H.R., 1998. Genetics of programmed cell death in C. elegans: past, present and future. Trends Genet. 14, 410–416. http://dx.doi.org/10.1016/S0168-9525(98)01573-X.

Miccoli, A., Gioacchini, G., Maradonna, F., Benato, F., Skobo, T., Carnevali, O., 2015. Beneficial Bacteria affect *Danio rerio* development by the modulation of maternal factors involved in autophagic, apoptotic and dorsalizing processes. Cell. Physiol. Biochem. 35, 1706–1718. http://dx.doi.org/10.1159/000373983.

Michlewski, G., Cáceres, J.F., 2010. Antagonistic role of hnRNP A1 and KSRP in the regulation of let-7a biogenesis. Nat. Struct. Mol. Biol. 17, 1011–1018. http://dx. doi.org/10.1038/nsmb.1874.

Michlewski, G., Guil, S., Cáceres, J.F., 2011. Stimulation of pri-miR-18a processing by hnRNP A1. Adv. Exp. Med. Biol. 700, 28–35. http://dx.doi.org/10.1007/978-1-4419-7823-3_3.

Mishima, Y., Giraldez, A.J., Takeda, Y., Fujiwara, T., Sakamoto, H., Schier, A.F., Inoue, K., 2006. Differential regulation of germline mRNAs in soma and germ cells by zebrafish miR-430. Curr. Biol. 16, 2135–2142. http://dx.doi.org/10.1016/j. cub.2006.08.086.

Mizuno, T., Yamaha, E., Kuroiwa, A., Takeda, H., 1999. Removal of vegetal yolk causes dorsal deficiencies and impairs dorsal-inducing ability of the yolk cell in zebrafish. Mech. Dev. 81, 51–63. http://dx.doi.org/10.1016/S0925-4773(98)00202-0.

Mizushima, N., Komatsu, M., 2011. Autophagy: renovation of cells and tissues. Cell 147, 728–741. http://dx.doi.org/10.1016/j.cell.2011.10.026.

Mizushima, N., Sugita, H., Yoshimori, T., Ohsumi, Y., 1998. A new protein conjugation system in human. The counterpart of the yeast Apg12p conjugation system essential for autophagy. J. Biol. Chem. 273, 33889–33892. http://dx.doi.org/10.1074/jbc.273.51.33889.

Mizushima, N., Kuma, A., Kobayashi, Y., Yamamoto, A., Matsubae, M., Takao, T., Natsume, T., Ohsumi, Y., Yoshimori, T., 2003. Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12–Apg5 conjugate. J. Cell Sci. 116, 1679–1688. http://dx.doi.org/10.1242/jcs.00381.

Mizushima, N., Yoshimori, T., Ohsumi, Y., 2011. The role of Atg proteins in autophagosome formation. Annu. Rev. Cell Dev. Biol. 27, 107–132. http://dx.doi. org/10.1146/annurev-cellbio-092910-154005.

Morris, E.J., Geller, H.M., 1996. Induction of neuronal apoptosis by camptothecin, an inhibitor of DNA topoisomerase-I: evidence for cell cycle-independent toxicity. J. Cell Biol. 134, 757–770. http://dx.doi.org/10.1083/jcb.134.3.757.

Muggenhumer, D., Vesely, C., Nimpf, S., Tian, N., Yongfeng, J., Jantsch, M.F., 2014. Drosha protein levels are translationally regulated during *Xenopus* oocyte maturation. Mol. Biol. Cell 25, 2094–2104. http://dx.doi.org/10.1091/mbc.E13- 07-0386.

Muraoka, O., Shimizu, T., Yabe, T., Nojima, H., Bae, Y.-K., Hashimoto, H., Hibi, M., 2006. Sizzled controls dorso-ventral polarity by repressing cleavage of the Chordin protein. Nat. Cell Biol. 8, 329–338. http://dx.doi.org/10.1038/ncb1379.

Nazio, F., Strappazzon, F., Antonioli, M., Bielli, P., Cianfanelli, V., Bordi, M., Gretzmeier, C., Dengjel, J., Piacentini, M., Fimia, G.M., Cecconi, F., 2013. MTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. Nat. Cell Biol. 15, 406–416. http://dx.doi.org/10.1038/ncb2708.

Negron, J.F., Lockshin, R.a., 2004. Activation of apoptosis and caspase-3 in zebrafish early gastrulae. Dev. Dyn. 231, 161–170. http://dx.doi.org/10.1002/dvdy.20124. Nepal, C., Hadzhiev, Y., Previti, C., Haberle, V., Li, N., Takahashi, H., Suzuki, A.M.M., Sheng, Y., Abdelhamid, R.F., Anand, S., Gehrig, J., Akalin, A., Kockx, C.E.M., Van

Der Sloot, A.a.J., Van IJcken, W.F.J., Armant, O., Rastegar, S., Watson, C., Strahle, U., Stupka, E., Carninci, P., Lenhard, B., Muller, F., 2013. Dynamic regulation of the transcription initiation landscape at single nucleotide resolution during vertebrate embryogenesis. Genome Res. 23, 1938–1950. http://dx.doi.org/10.1101/gr.153692.112.

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. http://dx.doi.org/10.1016/0092-8674(82)90272-0.

Newport, J., Kirschner, M., 1982b. A major developmental transition in early *Xenopus* embryos: II. Control of the onset of transcription. Cell 30, 687–696. http://dx.doi.org/10.1016/0092-8674(82)90273-2.

Niehrs, C., Keller, R., Cho, K.W., De Robertis, E.M., 1993. The homeobox gene goosecoid controls cell migration in *Xenopus* embryos. Cell 72, 491–503. http:// dx.doi.org/10.1016/0092-8674(93)90069-3.

Nikaido, M., Tada, M., Takeda, H., Kuroiwa, A., Ueno, N., 1999. In vivo analysis using variants of zebrafish BMPR-IA: range of action and involvement of BMP in ectoderm patterning. Development 126, 181–190.

Nishida, H., 2005. Specification of embryonic axis and mosaic development in ascidians. Dev. Dyn. 233, 1177–1193. http://dx.doi.org/10.1002/dvdy.20469.

Norbury, C.J., Hickson, I.D., 2001. Cellular responses to DNA damage. Annu. Rev. Pharmacol. Toxicol. 41, 367–401. http://dx.doi.org/10.1146/annurev. pharmtox.41.1.367.

Ober, E.a, Schulte-Merker, S., 1999. Signals from the yolk cell induce mesoderm, neuroectoderm, the trunk organizer, and the notochord in zebrafish. Dev. Biol. 215, 167–181. http://dx.doi.org/10.1006/dbio.1999.9455.

Oelgeschläger, M., Larraín, J., Geissert, D., De Robertis, E.M., 2000. The evolutionarily conserved BMP-binding protein Twisted gastrulation promotes BMP signalling. Nature 405, 757–763. http://dx.doi.org/10.1038/35015500.

Oelgeschläger, M., Kuroda, H., Reversade, B., De Robertis, E.M., 2003. Chordin is required for the spemann organizer transplantation phenomenon in *Xenopus* embryos. Dev. Cell 4, 219–230. http://dx.doi.org/10.1016/S1534-5807(02)00404-5.

Ohsumi, Y., 2006. Protein turnover. IUBMB Life 58, 363–369. http://dx.doi.org/10.1080/15216540600758539.

Ohsumi, Y., 2014. Historical landmarks of autophagy research. Cell Res. 24, 9–23. http://dx.doi.org/10.1038/cr.2013.169.

Okuda, Y., Ogura, E., Kondoh, H., Kamachi, Y., 2010. B1 SOX coordinate cell specification with patterning and morphogenesis in the early zebrafish embryo. PLoS Genet. 6, e1000936. http://dx.doi.org/10.1371/journal.pgen.1000936.

Onichtchouk, D., Geier, F., Polok, B., Messerschmidt, D.M., Mössner, R., Wendik, B., Song, S., Taylor, V., Timmer, J., Driever, W., 2010. Zebrafish Pou5f1-dependent transcriptional networks in temporal control of early development. Mol. Syst. Biol. 6, 354. http://dx.doi.org/10.1038/msb.2010.9.

Oppenheim, R.W., 1991. Cell death during development of the nervous system. Annu. Rev. Neurosci. 14, 453–501. http://dx.doi.org/10.1146/annurev. ne.14.030191.002321.

Orkin, S.H., Hochedlinger, K., 2011. Chromatin connections to pluripotency and cellular reprogramming. Cell 145, 835–850. http://dx.doi.org/10.1016/ j.cell.2011.05.019.

Pagliarini, V., Wirawan, E., Romagnoli, a., Ciccosanti, F., Lisi, G., Lippens, S., Cecconi, F., Fimia, G.M., Vandenabeele, P., Corazzari, M., Piacentini, M., 2012. Proteolysis of Ambra1 during apoptosis has a role in the inhibition of the autophagic pro- survival response. Cell Death Differ. 19, 1495–1504. http://dx.doi.org/10.1038/ cdd.2012.27.

Paillard, L., Osborne, H.B., 2003. East of EDEN was a poly(A) tail. Biol. Cell 95, 211–219, S0248490003000388 [pii].

Pattingre, S., Tassa, A., Qu, X., Garuti, R., Liang, X.H., Mizushima, N., Packer, M., Schneider, M.D., Levine, B., 2005. Bcl-2 antiapoptotic proteins inhibit Beclin 1- dependent autophagy. Cell 122, 927–939. http://dx.doi.org/10.1016/ j.cell.2005.07.002.

Paweletz, N., 2001. Walther Flemming: pioneer of mitosis research. Nat. Rev. Mol. Cell Biol. 2, 72–75. http://dx.doi.org/10.1038/35048077.

Pelegri, F., 2003. Maternal factors in zebrafish development. Dev. Dyn. 228, 535–554. http://dx.doi.org/10.1002/dvdy.10390.

Penaloza, C., Lin, L., Lockshin, R.a., Zakeri, Z., 2006. Cell death in development: shaping the embryo. Histochem. Cell Biol. 126, 149–158. http://dx.doi.org/10.1007/s00418-006-0214-1.

Piccolo, S., Sasai, Y., Lu, B., De Robertis, E.M., 1996. Dorsoventral patterning in *Xenopus*: inhibition of ventral signals by direct binding of chordin to BMP-4. Cell 86, 589–598. http://dx.doi.org/10.1016/S0092-8674(00)80132-4.

Piccolo, S., Agius, E., Lu, B., Goodman, S., Dale, L., De Robertis, E.M., 1997. Cleavage of chordin by xolloid metalloprotease suggests a role for proteolytic processing in the regulation of spemann organizer activity. Cell 91, 407–416. http://dx.doi. org/10.1016/S0092-8674(00)80424-9.

Piskounova, E., Polytarchou, C., Thornton, J.E., Lapierre, R.J., Pothoulakis, C., Hagan, J. P., Iliopoulos, D., Gregory, R.I., 2011. Lin28A and Lin28B inhibit let-7 MicroRNA biogenesis by distinct mechanisms. Cell 147, 1066–1079. http://dx.doi.org/10.1016/j.cell.2011.10.039.

Potok, M.E., Nix, D.a., Parnell, T.J., Cairns, B.R., 2013. Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern. Cell 153, 759–772. http://dx.doi.org/10.1016/j.cell.2013.04.030.

Prioleau, M.N., Huet, J., Sentenac, a., Méchali, M., 1994. Competition between chromatin and transcription complex assembly regulates gene expression during early development. Cell 77, 439–449. http://dx.doi.org/10.1016/0092-8674(94)90158-9.

Qu, X., Zou, Z., Sun, Q., Luby-Phelps, K., Cheng, P., Hogan, R.N., Gilpin, C., Levine, B., 2007. Autophagy gene-dependent clearance of apoptotic cells during embryonic development. Cell 128, 931–946. http://dx.doi.org/10.1016/ j.cell.2006.12.044.

Rai, K., Nadauld, L.D., Chidester, S., Manos, E.J., James, S.R., Karpf, A.R., Cairns, B.R., Jones, D.a., 2006. Zebra fish Dnmt1 and Suv39h1 regulate organ-specific terminal differentiation during development. Mol. Cell. Biol. 26, 7077–7085. http://dx.doi.org/10.1128/MCB.00312-06.

Rentzsch, F., Zhang, J., Kramer, C., Sebald, W., Hammerschmidt, M., 2006. Crossveinless 2 is an essential positive feedback regulator of Bmp signaling during zebrafish gastrulation. Development 133, 801–811. http://dx.doi.org/10.1242/dev.02250.

Reversade, B., Kuroda, H., Lee, H., Mays, A., De Robertis, E.M., 2005. Depletion of Bmp2, Bmp4, Bmp7 and Spemann organizer signals induces massive brain formation in *Xenopus* embryos. Development 132, 3381–3392. http://dx.doi. org/10.1242/dev.01901.

Richter, J.D., 1999. Cytoplasmic polyadenylation in development and beyond. Microbiol. Mol. Biol. Rev. 63, 446–456.

Richter, J.D., Lasko, P., 2011. Translational control in oocyte development. Cold Spring Harb. Perspect. Biol. 3, a002758. http://dx.doi.org/10.1101/cshperspect. a002758.

Rivera-Pérez, J.a., Mallo, M., Gendron-Maguire, M., Gridley, T., Behringer, R.R., 1995. Goosecoid is not an essential component of the mouse gastrula organizer but is required for craniofacial and rib development. Development 121, 3005–3012.

Rodriguez, M., Driever, W., 1997. Mutations resulting in transient and localized degeneration in the developing zebrafish brain. Biochem. Cell Biol. 75, 579–600. Rollo, a., Sulpizio, R., Nardi, M., Silvi, S., Orpianesi, C., Caggiano, M., Cresci, a., Carnevali, O., 2006. Live microbial feed supplement in aquaculture for improvement of stress tolerance. Fish Physiol. Biochem. 32, 167–177. http://dx.doi.org/10.1007/s10695-006-0009-2.

Rosa, A., Brivanlou, A.H., 2009. MicroRNAs in early vertebrate development. Cell Cycle 8, 3513–3520. http://dx.doi.org/10.4161/cc.8.21.9847.

Rothstein, J.L., Johnson, D., DeLoia, J.A., Skowronski, J., Solter, D., Knowles, B., 1992. Gene expression during preimplantation mouse development. Genes Dev. 6, 1190–1201.

Ryu, S.L., Fujii, R., Yamanaka, Y., Shimizu, T., Yabe, T., Hirata, T., Hibi, M., Hirano, T.,2001. Regulation of dharma/bozozok by the Wnt pathway. Dev. Biol. 231, 397–409. http://dx.doi.org/10.1006/dbio.2000.0150.

Sanjuan, M.A., Dillon, C.P., Tait, S.W.G., Moshiach, S., Dorsey, F., Connell, S., Komatsu, M., Tanaka, K., Cleveland, J.L., Withoff, S., Green, D.R., 2007. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. Nature 450, 1253–1257. http://dx.doi.org/10.1038/nature06421.

Sasai, Y., Lu, B., Steinbeisser, H., Geissert, D., Gont, L.K., De Robertis, E.M., 1994. *Xenopus* chordin: a novel dorsalizing factor activated by organizer-specific homeobox genes. Cell 79, 779–790. http://dx.doi.org/10.1016/0092-8674(94)90068-X.

Sasai, Y., Lu, B., Steinbeisser, H., De Robertis, E.M., 1995. Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in *Xenopus*. Nature. http://dx.doi.org/10.1038/376333a0.

Saunders, A., Faiola, F., Wang, J., 2013. Concise review: pursuing self-renewal and pluripotency with the stem cell factor nanog. Stem Cells 31, 1227–1236. http:// dx.doi.org/10.1002/stem.1384.

Sawicki, J.A., Magnuson, T., Epstein, C.J., 1981. Evidence for expression of the paternal genome in the two-cell mouse embryo. Nature 294, 450–451.

Schier, a.F., 2001. Axis formation and patterning in zebrafish. Curr. Opin. Genet. Dev. 11, 393–404. http://dx.doi.org/10.1016/S0959-437X(00)00209-4.

Schneider, S., Steinbeisser, H., Warga, R.M., Hausen, P., 1996. b-Catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. Mech. Dev. 57, 191–198. http://dx.doi.org/10.1016/0925-4773(96)00546-1.

Schuettengruber, B., Ganapathi, M., Leblanc, B., Portoso, M., Jaschek, R., Tolhuis, B., van Lohuizen, M., Tanay, A., Cavalli, G., 2009. Functional anatomy of polycomb and trithorax chromatin landscapes in Drosophila embryos. PLoS Biol. 7, e13. http://dx.doi.org/10.1371/journal.pbio.1000013.

Schuff, M., Siegel, D., Philipp, M., Bundschu, K., Heymann, N., Donow, C., Knöchel, W., 2012. Characterization of *Danio rerio* Nanog and functional comparison to *Xenopus* vents. Stem Cells Dev. 21, 1225–1238. http://dx.doi.org/10.1089/ scd.2011.0285.

Schulte-Merker, S., Hammerschmidt, M., Beuchle, D., Cho, K.W., De Robertis, E.M., Nüsslein-Volhard, C., 1994. Expression of zebrafish goosecoid and no tail gene products in wild-type and mutant no tail embryos. Development 120, 843–852.

Shen, M.M., 2007. Nodal signaling: developmental roles and regulation. Development 134, 1023–1034. http://dx.doi.org/10.1242/dev.000166.

Shimizu, T., Yamanaka, Y., Ryu, S.L., Hashimoto, H., Yabe, T., Hirata, T., Bae, Y.K., Hibi, M., Hirano, T., 2000. Cooperative roles of Bozozok/Dharma and Nodal-related proteins in the formation of the dorsal organizer in zebrafish. Mech. Dev. 91, 293–303. http://dx.doi.org/10.1016/S0925-4773(99)00319-6.

Shimizu, T., Yamanaka, Y., Nojima, H., Yabe, T., Hibi, M., Hirano, T., 2002. A novel repressor-type homeobox gene, ved, is involved in dharma/bozozok-mediated dorsal organizer formation in zebrafish. Mech. Dev. 118, 125–138. http://dx.doi. org/10.1016/S0925-4773(02)00243-5.

Sirotkin, H.I., Dougan, S.T., Schier, A.F., Talbot, W.S., 2000. Bozozok and squint act in parallel to specify dorsal mesoderm and anterior neuroectoderm in zebrafish. Development 127, 2583–2592.

Skobo, T., Benato, F., Grumati, P., Meneghetti, G., Cianfanelli, V., Castagnaro, S., Chrisam, M., Di Bartolomeo, S., Bonaldo, P., Cecconi, F., Dalla Valle, L., 2014. Zebrafish ambra1a and ambra1b knockdown impairs skeletal muscle development. PLoS ONE 9, 1–13. http://dx.doi.org/10.1371/journal. pone.0099210.

St Johnston, D., Nüsslein-Volhard, C., 1992. The origin of pattern and polarity in the Drosophila embryo. Cell 68, 201–219. http://dx.doi.org/10.1016/0092-8674(92) 90466-P.

Stachel, S.E., Grunwald, D.J., Myers, P.Z., 1993. Lithium perturbation and goosecoid expression identify a dorsal specification pathway in the pregastrula zebrafish. Development 117, 1261–1274.

Stack, J.H., Newport, J.W., 1997. Developmentally regulated activation of apoptosis early in *Xenopus* gastrulation results in cyclin A degradation during interphase of the cell cycle. Development 124, 3185–3195.

Stancheva, I., Median, R.R., 2000. Transient depletion of xDnmt1 leads to premature gene activation in *Xenopus* embryos. Genes Dev. 14, 313–327. http://dx.doi.org/10.1101/gad.14.3.313.

Strappazzon, F., Vietri-Rudan, M., Campello, S., Nazio, F., Florenzano, F., Fimia, G.M., Piacentini, M., Levine, B., Cecconi, F., 2011. Mitochondrial BCL-2 inhibits AMBRA1-induced autophagy. EMBO J. 30, 1195–1208. http://dx.doi.org/10.1038/emboj.2011.49.

Strasser, A., Jost, P.J., Nagata, S., 2009. The many roles of FAS receptor signaling in the immune system. Immunity 30, 180–192. http://dx.doi.org/10.1016/j. immuni.2009.01.001.

Suzer, C., Çoban, D., Kamaci, H.O., Saka, S ., Firat, K., Otgucuog˘lu, Ö., Küçüksari, H., 2008. Lactobacillus spp. bacteria as probiotics in gilthead sea bream (Sparus aurata L.) larvae: effects on growth performance and digestive enzyme activities. Aquaculture 280, 140–145. http://dx.doi.org/10.1016/j. aquaculture.2008.04.020.

Tadros, W., Lipshitz, H.D., 2009. The maternal-to-zygotic transition: a play in two acts. Development 136, 3033–3042. http://dx.doi.org/10.1242/dev.033183.

Tait, S.W.G., Green, D.R., 2010. Mitochondria and cell death: outer membrane permeabilization and beyond. Nat. Rev. Mol. Cell Biol. 11, 621–632. http://dx. doi.org/10.1038/nrm2952.

Tang, D., Lahti, J.M., Grenet, J., Kidd, V.J., 1999. Cycloheximide-induced T-cell death is mediated by a Fas-associated death domain-dependent mechanism. J. Biol. Chem. 274, 7245–7252. http://dx.doi.org/10.1074/jbc.274.11.7245.

Tay, Y., Tam, W.-L., Ang, Y.-S., Gaughwin, P.M., Yang, H., Wang, W., Liu, R., George, J., Ng, H.-H., Perera, R.J., Lufkin, T., Rigoutsos, I., Thomson, A.M., Lim, B., 2008a. MicroRNA-134 modulates the differentiation of mouse embryonic stem cells, where it causes post-transcriptional attenuation of Nanog and LRH1. Stem Cells 26, 17–29. http://dx.doi.org/10.1634/stemcells.2007-0295.

Tay, Y., Zhang, J., Thomson, A.M., Lim, B., Rigoutsos, I., 2008b. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 455, 1124–1128. http://dx.doi.org/10.1038/nature07299.

Thisse, B., Thisse, C., 2005. Functions and regulations of fibroblast growth factor signaling during embryonic development. Dev. Biol. 287, 390–402. http://dx. doi.org/10.1016/j.ydbio.2005.09.011.

Tittle, R.K., Sze, R., Ng, A., Nuckels, R.J., Swartz, M.E., Anderson, R.M., Bosch, J., Stainier, D.Y.R., Eberhart, J.K., Gross, J.M., 2011. Uhrf1 and Dnmt1 are required for development and maintenance of the zebrafish lens. Dev. Biol. 350, 50–63. http://dx.doi.org/10.1016/j.ydbio.2010.11.009.

Trede, N.S., Langenau, D.M., Traver, D., Look, a.T., Zon, L.I., 2004. The use of zebrafish to understand immunity. Immunity 20, 367–379. http://dx.doi.org/10.1016/ S1074-7613(04)00084-6.

Ulitsky, I., Shkumatava, A., Jan, C.H., Subtelny, A.O., Koppstein, D., Bell, G.W., Sive, H., Bartel, D.P., 2012. Extensive alternative polyadenylation during zebrafish development. Genome Res. 22, 2054–2066. http://dx.doi.org/10.1101/ gr.139733.112.

Valencia, C.A., Bailey, C., Liu, R., 2007. Novel zebrafish caspase-3 substrates. Biochem. Biophys. Res. Commun. 361, 311–316. http://dx.doi.org/10.1016/j. bbrc.2007.06.173.

Van Es, J.H., Barker, N., Clevers, H., 2003. You Wnt some, you lose some: oncogenes in the Wnt signaling pathway. Curr. Opin. Genet. Dev. 13, 28–33. http://dx.doi. org/10.1016/S0959-437X(02)00012-6.

Van Wynsberghe, P.M., Kai, Z.S., Massirer, K.B., Burton, V.H., Yeo, G.W., Pasquinelli, A.E., 2011. LIN-28 co-transcriptionally binds primary let-7 to regulate miRNA maturation in Caenorhabditis elegans. Nat. Struct. Mol. Biol. 18, 302–308. http:// dx.doi.org/10.1038/nsmb.1986.

Vastenhouw, N.L., Zhang, Y., Woods, I.G., Imam, F., Regev, A., Liu, X.S., Rinn, J., Schier, A.F., 2010. Chromatin signature of embryonic pluripotency is established during genome activation. Nature 464, 922–926. http://dx.doi.org/10.1038/nature08866.

Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W., 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev. 64, 655–671. http://dx.doi.org/10.1128/MMBR.64.4.655-671.2000.Updated.

Villalba, A., Coll, O., Gebauer, F., 2011. Cytoplasmic polyadenylation and translational control. Curr. Opin. Genet. Dev. 21, 452–457. http://dx.doi.org/10.1016/j.gde.2011.04.006.

Wagner, D.S., Mullins, M.C., 2002. Modulation of BMP activity in dorsal-ventral pattern formation by the chordin and ogon antagonists. Dev. Biol. 245, 109–123. http://dx.doi.org/10.1006/dbio.2002.0614.

Wang, J., Lenardo, M.J., 2000. Roles of caspases in apoptosis, development, and cytokine maturation revealed by homozygous gene deficiencies. J. Cell Sci. 113 (Pt. 5), 753–757.

Wang, Q.T., Piotrowska, K., Ciemerych, M.A., Milenkovic, L., Scott, M.P., Davis, R.W., Zernicka-Goetz, M., 2004. A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo. Dev. Cell 6, 133–144. http://dx.doi.org/10.1016/S1534-5807(03)00404-0.

Wang, J., Rao, S., Chu, J., Shen, X., Levasseur, D.N., Theunissen, T.W., Orkin, S.H., 2006. A protein interaction network for pluripotency of embryonic stem cells. Nature 444, 364–368. http://dx.doi.org/10.1038/nature05284.

Watabe, T., Kim, S., Candia, a., Rothbächer, U., Hashimoto, C., Inoue, K., Cho, K.W., 1995. Molecular mechanisms of Spemann's organizer formation: conserved growth factor synergy between *Xenopus* and mouse. Genes Dev. 9, 3038–3050. http://dx.doi.org/10.1101/gad.9.24.3038.

Watanabe, T., Takeda, A., Mise, K., Okuno, T., Suzuki, T., Minami, N., Imai, H., 2005. Stage-specific expression of microRNAs during *Xenopus* development. FEBS Lett. 579, 318–324. http://dx.doi.org/10.1016/j.febslet.2004.11.067.

Wei, Z., Angerer, R.C., Angerer, L.M., 2006. A database of mRNA expression patterns for the sea urchin embryo. Dev. Biol. 300, 476–484. http://dx.doi.org/10.1016/j. ydbio.2006.08.034.

Wienholds, E., Koudijs, M.J., van Eeden, F.J.M., Cuppen, E., Plasterk, R.H.A., 2003. The microRNA-producing enzyme Dicer1 is essential for zebrafish development. Nat. Genet. 35, 217–218. http://dx.doi.org/10.1038/ng1251.

Wilson, R.C., Doudna, J.A., 2013. Molecular mechanisms of RNA interference. Annu.

Rev. Biophys. 42, 217–239. http://dx.doi.org/10.1146/annurev-biophys-083012-130404.

Wilson, N.S., Dixit, V., Ashkenazi, A., 2009. Death receptor signal transducers: nodes of coordination in immune signaling networks. Nat. Immunol. 10, 348–355. http://dx.doi.org/10.1038/ni.1714.

Wirawan, E., Vande Walle, L., Kersse, K., Cornelis, S., Claerhout, S., Vanoverberghe, I., Roelandt, R., De Rycke, R., Verspurten, J., Declercq, W., Agostinis, P., Vanden Berghe, T., Lippens, S., Vandenabeele, P., 2010. Caspase-mediated cleavage of Beclin-1 inactivates Beclin-1-induced autophagy and enhances apoptosis by promoting the release of proapoptotic factors from mitochondria. Cell Death Dis. 1, e18. http://dx.doi.org/10.1038/cddis.2009.16.

Wirawan, E., Lippens, S., Vanden Berghe, T., Romagnoli, A., Fimia, G.M., Piacentini, M., Vandenabeele, P., 2012. Beclin1: a role in membrane dynamics and beyond. Autophagy 8, 6–17. http://dx.doi.org/10.4161/auto.8.1.16645.

Wulff, B.-E., Nishikura, K., 2012. Modulation of microRNA expression and function by ADARs. Curr. Top. Microbiol. Immunol. 353, 91–109. http://dx.doi.org/10.1007/82_2011_151.

Xie, J., Fisher, S., 2005. Twisted gastrulation enhances BMP signaling through chordin dependent and independent mechanisms. Development 132, 383–391. http://dx.doi.org/10.1242/dev.01577.

Xu, C., Fan, Z.P., Müller, P., Fogley, R., DiBiase, A., Trompouki, E., Unternaehrer, J.,Xiong, F., Torregroza, I., Evans, T., Megason, S.G., Daley, G.Q., Schier, A.F., Young, R.a., Zon, L.I., 2012. Nanog-like regulates endoderm formation through the Mxtx2-Nodal pathway. Dev. Cell 22, 625–638. http://dx.doi.org/10.1016/j. devcel.2012.01.003.

Yabe, T., Ge, X., Pelegri, F., 2007. The zebrafish maternal-effect gene cellular atoll encodes the centriolar component sas-6 and defects in its paternal function promote whole genome duplication. Dev. Biol. 312, 44–60. http://dx.doi.org/10.1016/j.ydbio.2007.08.054.

Yabu, T., Kishi, S., Okazaki, T., Yamashita, M., 2001. Characterization of zebrafish caspase-3 and induction of apoptosis through ceramide generation in fish fathead minnow tailbud cells and zebrafish embryo. Biochem. J. 360, 39–47. http://dx.doi.org/10.1042/0264-6021:3600039.

Yamada, G., Mansouri, A., Torres, M., Stuart, E.T., Blum, M., Schultz, M., Robertis, E.M.

De, Gruss, P., De Robertis, E.M., 1995. Targeted mutation of the murine goosecoid gene results in craniofacial defects and neonatal death. Development 121, 2917–2922.

Yamashita, M., 2003. Apoptosis in zebrafish development. Comp. Biochem. Physiol.– B Biochem. Mol. Biol. 136, 731–742. http://dx.doi.org/10.1016/j. cbpc.2003.08.013.

Youle, R.J., Strasser, A., 2008. The BCL-2 protein family: opposing activities that mediate cell death. Nat. Rev. Mol. Cell Biol. 9, 47–59. http://dx.doi.org/10.1038/ nrm2308.

Yue, Z., Jin, S., Yang, C., Levine, A.J., Heintz, N., 2003. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc. Natl. Acad. Sci. U.S.A. 100, 15077–15082. http://dx.doi.org/10.1073/pnas.2436255100.

Zaret, K.S., Carroll, J.S., 2011. Pioneer transcription factors: establishing competence for gene expression. Genes Dev. 25, 2227–2241. http://dx.doi.org/10.1101/gad.176826.111.

Zhu, H., Wu, H., Liu, X., Li, B., Chen, Y., Ren, X., Liu, C.-G., Yang, J.-M., 2009. Regulation of autophagy by a beclin 1-targeted microRNA, miR-30a, in cancer cells. Autophagy 5, 816–823, 9064.