Review

Mitochondria, redox signaling and axis specification in metazoan embryos

James A. Coffman [a],*, James M. Denegre [b],*

[a] Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672, USA
[b] The Jackson Laboratory, Bar Harbor, ME 04609, USA

Abstract

Mitochondria are not only the major energy generators of the eukaryotic cell but they are also sources of signals that control gene expression and cell fate. While mitochondria are often asymmetrically distributed in early embryos, little is known about how they contribute to axial patterning. Here we review studies of mitochondrial distribution in metazoan eggs and embryos and the mechanisms of redox signaling, and speculate on the role that mitochondrial anisotropies might play in the developmental specification of cell fate during embryogenesis of sea urchins and other animals.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Mitochondria; Embryo; Polarity; Redox state; Hypoxia; Spatial differentiation; Transcription factors; Kinase; Phosphatase; Cytoskeleton

Introduction

Much of the energy that sustains animal life is generated by redox chemistry carried out by mitochondria, modern descendents of bacterial endosymbionts that took up residence within our primordial eukaryotic ancestors. Although these organelles were long regarded as little more than the "powerhouses" of the cell, recent discoveries have revealed that they are also a source of biological signals that control cell fate. In addition to their now famous role in the initiation of apoptosis, mitochondria can affect gene expression by modulating intracellular calcium dynamics and by emitting reactive oxygen species (ROS) that serve as signaling intermediates. They therefore appear to be intimately involved in the flow of both energy and information, the two defining attributes of life.

An intellectual legacy of molecular biology's ascent during the last half of the twentieth century is that ontogeny is now generally regarded as being genetically determined or "programmed". While the genetic control of development is undeniable, providing a paradigm that guides most current research in developmental biology, the fact remains that gene activity is itself governed by physiological context that includes environmental factors. The physiological/environmental control of development was once more widely appreciated, and was the major theme in the research program of Charles Manning Child, a prominent developmental biologist in the early twentieth century. Child's work was eventually eclipsed by that of his chief rival and embryologist-turned-geneticist Thomas Hunt Morgan, whose genocentric research paradigm using *Drosophila melanogaster* was buoyed by the molecular revolution (Blackstone, 2006). Whereas Morgan ultimately championed the view that development is controlled by genetic information contained within chromosomes, Child sought to establish the locus of developmental control in energy metabolism. The research of Child and colleagues in a wide variety of organisms showed that developmental potential and spatial patterning correlate with, and can sometimes be entrained by, gradients in metabolic rate (Child, 1941).

The two principal methods employed by Child for detecting metabolic gradients were differential staining with redox sensitive dyes (Figs. 1a, b) and differential susceptibility to metabolic poisons (Fig. 1c; Child, 1941). While Child did not specifically relate his theory to mitochondria, some of the dyes that he employed (such as janus green and indophenol blue) are now known to be mitochondrial stains (e.g., the indophenol blue

* Corresponding authors.
E-mail addresses: jcoffman@mdibl.org (J.A. Coffman),
james.denegre@jax.org (J.M. Denegre).

reaction is catalyzed by cytochrome oxidase; Czihak, 1963), and likewise, many of the metabolic poisons that he used (e.g. cyanide, azide) are specific inhibitors of mitochondrial respiration. At the same time, a number of Child's contemporaries (including Morgan) used centrifugal stratification of eggs to investigate the relationship of cytoplasmic constituents of the egg to embryonic polarities (e.g. Morgan and Lyon, 1907; Morgan and Spooner, 1909). Ethel Browne Harvey used vital dyes to visualize mitochondria and other organelles in the stratified sea urchin egg (Fig. 1d; Harvey, 1941), and Lindahl (1932) and Pease (1939) showed a correlation between the axis of centrifugation and the secondary (oral–aboral) axis of embryos developed from stratified sea urchin and sand dollar eggs. While these experiments did not specifically address the role of mitochondria in embryonic development, they can be interpreted retrospectively in this context; moreover, recent work has used centrifugal stratification as a tool to investigate the role of mitochondria in various aspects of development (e.g., Speksnijder, 1992; Coffman et al., 2004; Van Blerkom and Davis, 2006).

Two criticisms that led to ostensible demise of Child's theory were that his results did not definitively distinguish whether metabolic gradients were a 'cause' or 'effect' of development (Blackstone, 2000, 2006), and that metabolism is too general a phenomenon to account for the specific patterns generated during animal development. It can be argued that the former criticism stems from a simplistic notion of linear causation that does not realistically model biology, which is replete with autocatalytic cycles, feedback circuitry and other manifestations of circular causality (Coffman, 2006). The second criticism can be addressed by considering the nature of developmental robustness (Nijhout, 2002). Robustness (also known as canalization), arises as a result of non-linearities, for example in a transcriptional response to a diffusely graded signal which produces sharp spatial boundaries by virtue of cooperative DNA binding and the use of repressors within *cis*-regulatory modules (Davidson, 2006; Veitia and Nijhout, 2006). An example of this phenomenon is the deployment of Bicoid in a gradient along the anterior–posterior axis of the *Drosophila* embryo. This gradient displays a substantial amount of variability among embryos as well as sensitivity to environmental variables such as temperature, but this noise is ultimately removed by regulatory interactions downstream of Bicoid (Houchmandzadeh et al., 2002). The reaction–diffusion dynamics originally proposed by Alan Turing is another non-linear process whereby precise heterogeneous patterns can develop from noisy and more homogeneous conditions (Turing, 1952). Developmental patterning by Nodal and its antagonist Lefty is a real-life example of such a system (Solnica-Krezel, 2003). Despite growing appreciation for the roles played by non-linearity and robustness in developmental systems, Child's gradients are still generally considered to be an "epiphenomenon" lacking causal agency, and likewise metabolism, once a central focus of cell and developmental biology, is commonly viewed as a "housekeeping" process devoid of developmental information.

The revelation that mitochondria are a nexus controlling the flow of both energy and information invites us to revisit Child's school of thought, and ask if it contained a baby that was thrown out with the bathwater. Indeed, the groundbreaking studies of Neil Blackstone and colleagues on colonial hydroids indicate that redox signaling controls colony development in that cnidarian (Blackstone, 2000, 2003; Blackstone et al., 2005), while research in mammalian cell culture shows that redox state can affect the fate of cells derived from various tissues (e.g., Smith et al., 2000; Connor et al., 2005). Moreover, recent work suggests that a mitochondrial redox gradient contributes to axis specification in sea urchin embryos (Coffman and Davidson, 2001; Coffman et al., 2004). Finally, from the perspective of thermodynamics, it can be argued that energy gradients (and consequent flow) are a formal cause of development, which should motivate inquiry into how metabolism might entrain the material and efficient causes embodied by genes and their products (Coffman, 2006). Here we review what is known about mitochondrial distribution in animal embryos and the mechanisms of redox signaling, and speculate on how mitochondrial redox signaling might contribute to axial patterning of early embryos.

Distribution of mitochondria in eggs and embryos correlates with axial polarities

Mitochondrial distribution in ascidian embryos

Developmental patterning of the embryo is often presaged by the spatial arrangement of cytoplasmic constituents in the egg and zygote, as is most apparent in embryos that undergo a highly determinative or "mosaic" type of development. Ascidian embryos provide a particularly illustrative example, as classically exemplified by the work of Conklin (1905), who showed that the maternal yellow myoplasm that is localized along the animal–vegetal (AV) axis of the *Steyla* egg segregates to the future muscle cell lineage. This and other classical cell lineage studies led to the idea that pattern formation follows the localization of maternal cytoplasmic determinants (reviewed in Davidson, 1986). While such determinants are now typically considered to be regulatory mRNAs (e.g. Jeffery, 1988; Sardet et al., 2005) and proteins (e.g. Denegre et al., 1997), it is clear that organelles such as mitochondria and the endoplasmic reticulum (ER) are also localized in the egg in patterns relevant to axis specification, and that this localization correlates with dye reduction gradients observed by Child (1941) (Fig. 1a). Visualizations of mitochondrial movements in the ascidian egg and embryo have been obtained by staining live eggs with the vital dyes Janus green (Reverbari, 1956) and the fluorescent $DiOC_2$ (Zalokar and Sardet, 1984). These studies showed that the mitochondria essentially follow the same pattern of localization and segregation as the myoplasm. Further characterization has recently defined the myoplasm as a subcortical cytoplasmic domain rich in mitochondria, actin and intermediate filaments, and poor in endoplasmic reticulum (ER) and microtubules (Roegiers et al., 1999).

In the unfertilized egg of the ascidian *Phallusia*, the great majority of the mitochondria are distributed in the vegetal half, in a bowl-shaped subcortical domain (Reverbari, 1956; Zalokar

and Sardet, 1984; Roegiers et al., 1999). After fertilization, a vigorous cortical contraction causes the mitochondria to translocate to the posterior as the egg elongates slightly and forms a transient protrusion at the vegetal pole, called the contraction pole. The mitochondria are centered on the contraction pole, and the geometry of this organization predicts
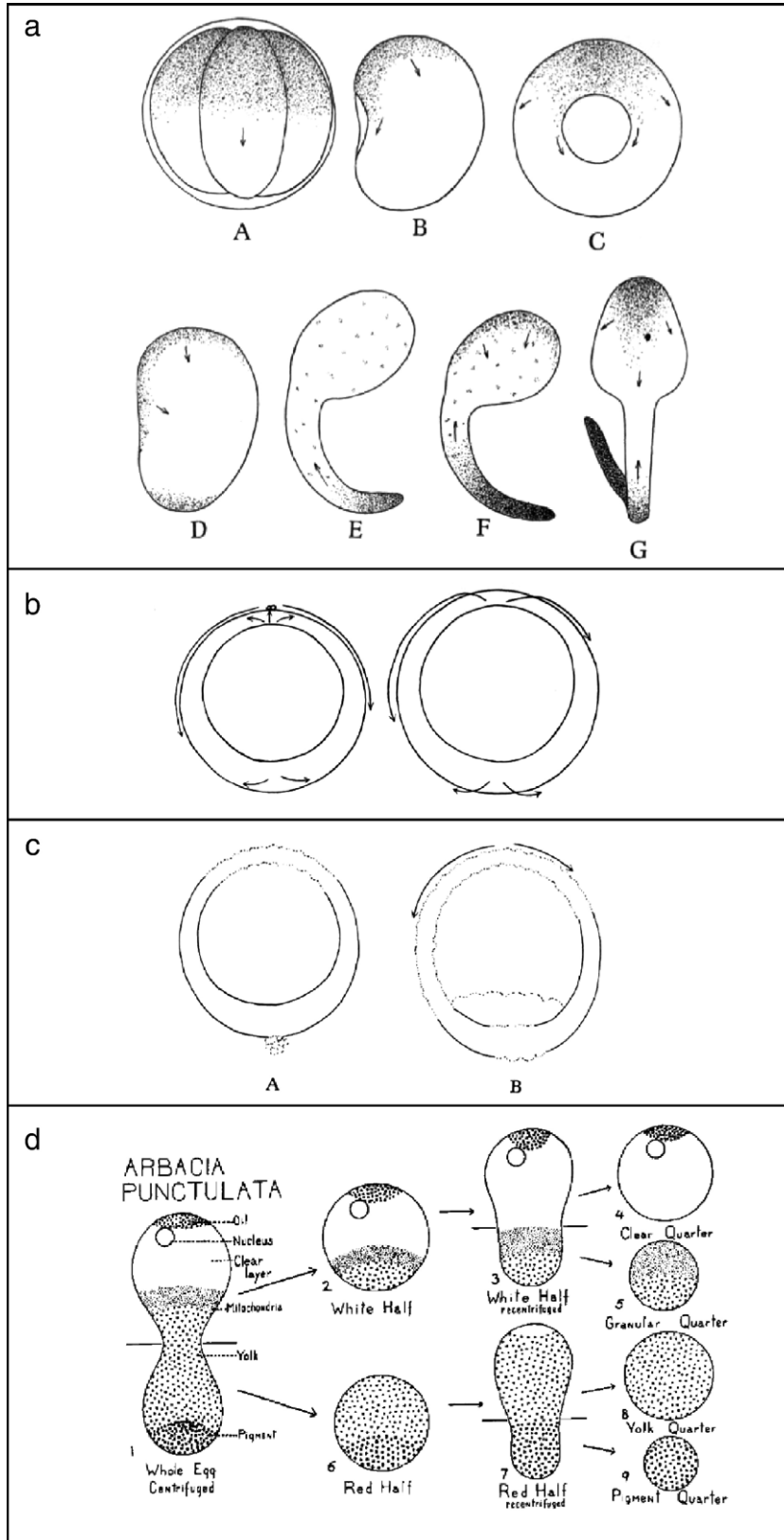
the patterning of the embryo's dorsal side and site of gastrulation (Roegiers et al., 1995). This initial translocation event strongly aggregates mitochondria to the vegetal pole, allowing for proper positioning in the zygote during subsequent translocations.

Prior to the next set of translocations another transient protrusion appears at the vegetal pole of the ascidian zygote, called the vegetal button. This structure contains cortical ER and a "nuage"-like granular domain thought to be germ plasm (nuage is a dense non-membrane-bound organelle typically associated with the germline). Interestingly, the vegetal button appears to exclude the subcortical mitochondria of the myoplasm. As the vegetal button regresses, the myoplasmic mitochondrial domain folds back upon itself and begins to translocate. The bulk of the mitochondria move to the future posterior pole while the remainder stay on the future anterior side (Roegiers et al., 1999). The result of these movements is a subequatorial mass of involuted mitochondria centered on the future posterior pole, and a smaller mitochondria-rich domain on the future anterior side. From the exterior, the mitochondrion is seen as a transverse stripe around the egg (Conklin, 1905; Reverbari, 1956; Roegiers et al., 1999).

During first cleavage, the mitochondria are equally distributed between the two blastomeres, and at the next two cleavages the majority of the mitochondria become segregated to the vegetal, posterior blastomeres (Reverbari, 1956; Zalokar and Sardet, 1984; Roegiers et al., 1999). Historically, these blastomeres are recognized as being specified for the posterior muscle cells of the tadpole (Conklin, 1905). A subset of the myoplasmic mitochondria, from the domain at the future anterior side of the egg, segregates to the anterior vegetal and posterior animal blastomeres.

It is clear that Conklin (Conklin, 1931) and subsequent investigators (Jeffery and Swalla, 1990; Satoh et al., 1996) considered the myoplasm not to be the muscle cell determinant per se, but rather as a co-segregating marker. Muscle cell determination and anteroposterior axis formation have been shown to depend on localized maternal mRNAs such as *macho-1* (Nishida and Sawada, 2001) and *posterior end mark* (PEM; Yoshida et al., 1996). Macho-1 and PEM belong to a class of maternal mRNAs named postplasmic/PEM RNAs (reviewed in Nishida, 2005; Sardet et al., 2005; Prodon et al., 2007; Sardet et al., 2007) which are initially localized along the AV axis of the oocyte during meiotic maturation (Prodon et al., 2006). At least some of the postplasmic/PEM RNAs are anchored to the cER, a distinct cortical ER domain between the sub-cortical mitochondrial myoplasm and the plasma membrane (Sardet et al., 2003; Prodon et al., 2005). After fertilization, the postplasmic/PEM

RNAs undergo a dramatic reorganization in a manner similar to the myoplasm, although there are differences in their final localizations, suggesting separate functions (reviewed in Sardet et al., 2005; Kumano and Nishida, 2007; Prodon et al., 2007; Sardet et al., 2007).

While localization of key mRNA determinants in the early embryo is well-established, it is often overlooked that the myoplasmic mitochondria also mark the future anterior–posterior (AP) axis and that regions of the egg containing the posterior mitochondria are important for axial patterning (Roegiers et al., 1999). The striking localization of mitochondria along the AP axis of the egg and embryo suggests that there may be a functional role of mitochondria in axial patterning that has not been considered and remains to be investigated (Dumollard et al., 2007a). Mitochondrial localizations may work in conjunction with localization of maternal mRNAs to regulate cell-specific gene activity.

Patterns of mitochondrial localization in various species

The localization of mitochondria in early development with respect to axis specification is a common feature for oocytes, eggs and embryos of many species. Although there are variations in the timing of when the localizations occur, they can be roughly grouped into three events: (1) a primary localization during oogenesis, (2) a secondary localization which results in the majority of mitochondria being tightly localized to one area of the egg or zygote, and (3) further movements resulting in aggregation and segregation of the mitochondria to subsets of blastomeres.

Primary localization

Mitochondrial anisotropies in many organisms appear to arise during oogenesis. For example, in early oogenesis in *Xenopus* mitochondria localize to the Balbiani body, a non-membranous structure containing mitochondria, ER, RNA, granular nuage, and proteins (reviewed in Guraya, 1979; Heasman et al., 1984; Tourte et al., 1984; Mignotte et al., 1987). The Balbiani body is a site of active mitochondrial DNA synthesis and mitochondriogenesis (Heasman et al., 1984; Tourte et al., 1984). An axis defined by the Balbiani body and bouquet-stage nucleus is presumed to be the initial AV axis of the oocyte, which becomes distinctly visible by the end of oogenesis (reviewed in Gerhart, 1980; King et al., 2005). After breakdown of the Balbiani body in early stage oocytes, the contents become localized to the cortex of the vegetal hemisphere (Heasman et al., 1984). This leads to the establishment of specific populations of mitochondria, germ plasm, RNAs and proteins in the vegetal half of the

Fig. 1. Historical antecedents for studies of the role of mitochondria in embryogenesis. (a, b) Dye reduction gradients observed by C.M. Child in (a) an ascidian (*Corella willmeriana*) embryo and (b) sea star (*Patiria*) blastulae. Arrows point from higher to lower rates of dye reduction (from Child, 1941). (c) Differential susceptibility of sea urchin (*Strongylocentrotus*) blastulae to anoxia or cyanide poisoning; cytolysis is indicated by stippling; arrows denote the progressive direction of cytolysis, with the longer arrows indicating faster rates (from Child, 1941). (d) Centrifugal stratification of vitally stained sea urchin (*Arbacia*) eggs by E.B. Harvey. Mitochondria stratify in a narrow zone toward the centrifugal pole, just above layers of yolk and pigment. The nucleus lies at the centripetal pole, just beneath a layer of oil droplets. Centrifugation of eggs with sufficient force causes them to separate into fragments, each containing a subset of the egg's cytoplasmic constituents (from Harvey, 1941; Reprinted with permission from the Marine Biological Laboratory, Woods Hole, MA).

oocyte (Heasman et al., 1984; Denegre et al., 1997; Mowry and Cote, 1999; Volodina et al., 2003), elaborating the initial AV polarity of the cell. A key aspect of AV axis specification is the localization of maternal mRNAs. Some maternal mRNAs become localized to the Balbiani body in *Xenopus* through the "early pathway", being trapped in the ER of the Balbiani body (Chang et al., 2004; reviewed in King et al., 2005); eventually relocalizing to the vegetal cortex during breakdown. In a manner similar to cER localization of postplasmic/PEM mRNAs, Balbiani body breakdown also leads to localization of mRNAs such as Veg1 in an ER-based active manner in the "late pathway" (Deshler et al., 1997; reviewed in King et al., 2005). In a visually distinctive organization termed the wedge, ER is seen to be layered, in cross section, in a wedge shape between the GV and the vegetal pole (reviewed in King et al., 2005). This formation is responsible for ER-dependent localization of mRNAs. The wedge also contains large amounts of mitochondria in a pattern similar to the ER (Denegre et al., 1997), and its location and behavior during Balbiani body breakdown would suggest a functional role in AV axis formation. Potential interactions between ER and mitochondria in the wedge have never been investigated, but there is ample evidence that mitochondria and ER have a defined physiology with consequences on signaling through calcium (Dumollard et al., 2006; Sardet et al., 2007).

A structure similar to the Balbiani body, the sponge body, is present in the *Drosophila* nurse cells and oocytes and is aligned with the oocyte nucleus along the AP axis, prior to nucleus migration. The sponge body is enriched in mitochondria, RNA and nuage and is associated with transport and localization of maternal components in the oocyte. The Balbiani body appears to be a common feature of oogenesis from insects to mammals, at least at the morphological level (Hertig, 1968; Hertig and Adams, 1967; Guraya, 1979; Jaglarz et al., 2003; Pepling et al., 2007; Figs. 2a, b). Although a molecular description of Balbiani body function in mammals is lacking, it is clear that the result of Balbiani body breakdown in other organisms is an elaboration of the initial AV axis of the oocyte, setting up localizations of determinants for patterning of the embryo.
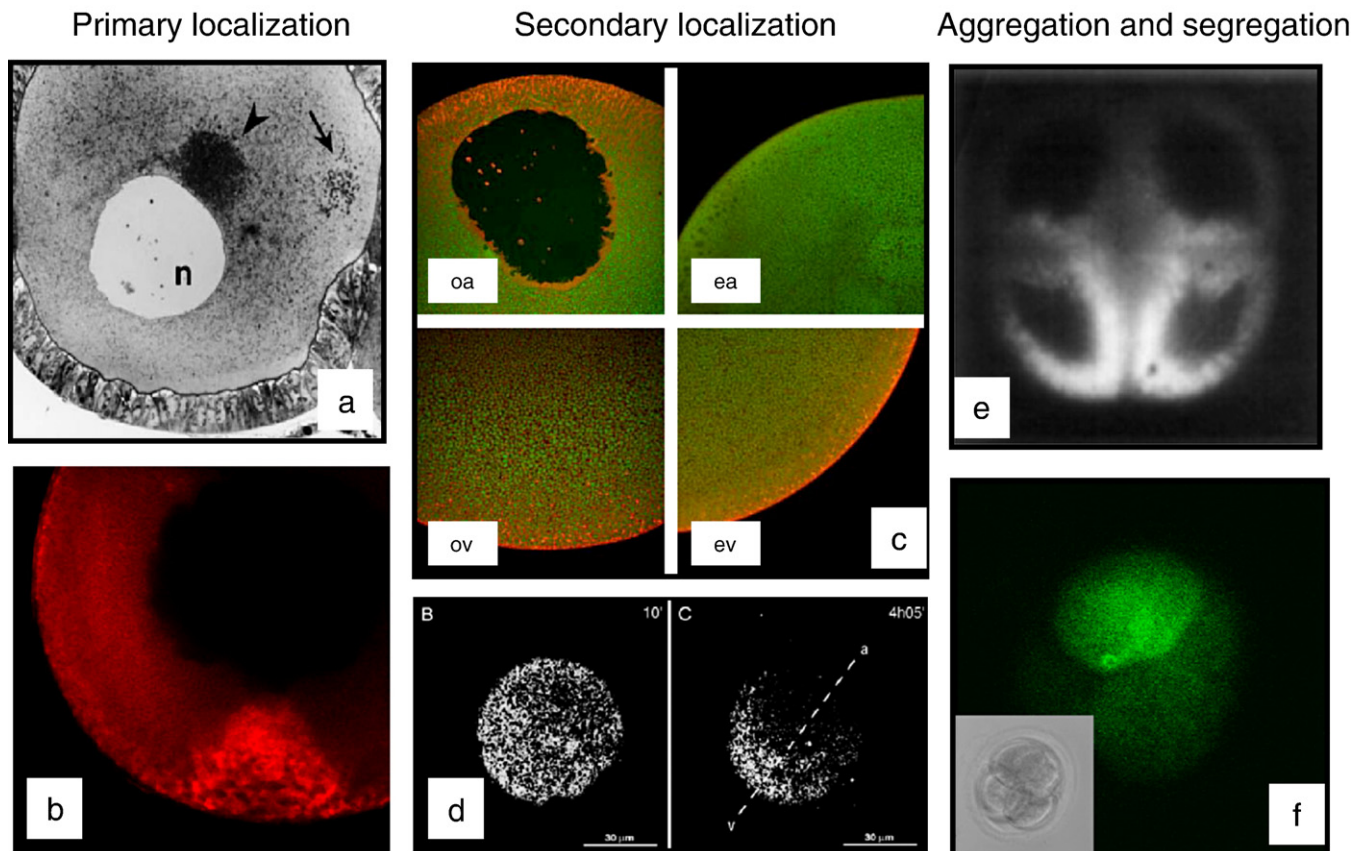

Fig. 2. Examples of the phases of mitochondrial localizations described in the text. (a, b) Primary localization in oocytes: (a) electron micrograph of oocyte of the tiger beetle (*Pseudoxycheila angustata*) showing the Balbiani body (arrowhead) adjacent to the nucleus. (b) Balbiani body breakdown and localization of mitochondria to the vegetal cortex in a Stage 3 oocyte of the frog *Xenopus*, visualized by anti-dihydrolipoamide acetyltransferase immunofluorescence. (c, d) Secondary localization during maturation: (c) Animal and vegetal views of stage VI *Xenopus* oocyte (oa/ov) and *in vitro* matured egg (ea/ev) visualized as in (b). Note the clearing of mitochondria from the animal hemisphere in the egg. (d) Distribution of mitochondria along the AV axis, visualized with the dye TMRE, prior to (left panel) and after (right panel) germinal vesicle breakdown in the ascidian *Ciona intestinalis*. (e, f) asymmetric segregation of mitochondria to blastomeres: (e) the 4-cell stage of *Phallusia mammillata* seen from the animal pole showing strong DiOC$_2$ fluorescence in the posterior cells. (f) 4-cell stage of *Mus muscus* showing strong MitoTracker Green™ fluorescence in one blastomere. Inset shows a transmitted light view of the embryo. Images courtesy of: (a) Jaglarz et al., 2003; (b, c) James Denegre; (d) Prodon et al., 2006; (e) Zalokar and Sardet, 1984; (f) James Denegre and Jacquelyn Masse.

The functional role of mitochondria in the initiation and elaboration of the AV axis is not clear, as it is a challenging process to investigate by experimentation; however, it was recently shown that genetic disruption of mitochondrial localization to the Balbiani body in *Drosophila* leads to developmental failure by the second instar stage (Cox and Spradling, 2006).

At the end of the primary translocation event in *Xenopus*, mitochondria are visible as reticulated, networked aggregates, or islands, in the vegetal subcortex, many of which are associated with germ plasm (Smith and Williams, 1975; Heasman et al., 1984; Savage and Danilchik, 1993; Wylie, 1999). The germ plasm is the germ line determinant (Illmensee and Mahowald, 1974, 1976; Illmensee et al., 1976) which consists of germinal granules embedded in aggregates of mitochondria. The germinal granules are poorly characterized electron-dense structures of RNA and proteins, thought to be directly related to nuage (Blackler, 1958; Smith, 1966; Mahowald, 1971a,b). Germ plasm is also found in cheatognaths, nematodes, ascidians, ctenophores and many insects (Eddy, 1975; Strome and Wood, 1982; Carre et al., 2002; Extavour and Akam, 2003), having in all cases a striking similarity in structure: it is a subcortical, electron-dense granular material, often embedded in mitochondria (although this is not the case in *Caenorhabditis elegans*). The *tud* gene product, which is essential for pole cell specification and polar granule formation, is found associated with both the germinal granules and the mitochondria of *Drosophila* (Bardsley et al., 1993). The functional significance of this association remains unclear.

Secondary localization

Not all of the mitochondria in the *Xenopus* oocyte are derived from the Balbiani body. Mitochondria active in DNA synthesis are present all around the germinal vesicle (Tourte et al., 1984; Mignotte et al., 1987). By the end of oogenesis, there is a reticulated network of mitochondria throughout all of the cytoplasm (Bement and Capco, 1990) which appear in the subcortex as islands in both the animal and vegetal hemispheres (Smith, 1966; Marchant et al., 2002). During the secondary localization event, these dispersed mitochondria become localized to the vegetal subcortex of the egg, presumably during maturation (Bement and Capco, 1990; Terasaki et al., 2001; Denegre and Mowry, unpublished; Figs. 2c, d). It is not known if secondary localization in *Xenopus* is an active movement of mitochondria, as in the ascidian *Phallusia* (Roegiers et al., 1999), or a remodeling of the mitochondria through breakdown. At fertilization, the majority of the mitochondria are localized in the subcortex in a bowl-shaped manner around the vegetal pole (Denegre et al., 1997; Volodina et al., 2003).

A secondary translocation during *in vitro* maturation of mouse oocytes has been described, where mitochondria translocate from cytoplasm to the periphery of the germinal vesicle (Van Blerkom, 1991). There is no strong consensus regarding mitochondrial localization at the end of maturation; the work of Calarco reports the mitochondria becoming almost entirely localized to the hemisphere containing the metaphase II spindle (Calarco, 1995), while others report that the mitochondria maintain a perinuclear localization (reviewed in Dumollard et al., 2007a). There is an absence of rigorous time-lapse image analysis of these events due to the sensitivity of mouse oocytes to fluorescent techniques for visualizing mitochondria and technical problems associated with culturing the oocytes (Denegre, personal observation; Dumollard et al., 2007a). During maturation, Van Blerkom (1991) noted a block in metaphase I spindle formation and failure of maturation when there was a low density of perinuclear mitochondria. Unlike secondary translocation in *Xenopus* and *Ciona*, in the mouse, the mitochondria are not localized to the cortex or subcortex, but are cytoplasmic. This difference might be related to the mode of axial patterning in the mouse, which is highly regulative and not dependent on maternal localizations of molecular determinants. Whether a pre-existing axial pattern is established via the cell biology of the mouse oocyte/egg is still an open and somewhat controversial question (Hiiragi et al., 2006; see below). The commonality in mammals of a secondary localization is difficult to assess because of the difficulty in maturing oocytes *in vitro*, and technical aspects in the fluorescent labeling of mitochondria (reviewed in Schatten et al., 2005; Dumollard et al., 2007a).

Aggregation and segregation

During the first cell cycle cortical contractions and cytoplasmic movements remodel localized mitochondria. In *Xenopus*, the mitochondrial islands and germ plasm in the vegetal cortex move with the yolk mass towards the sperm entry point and predicted ventral side during the cortical rotation (reviewed in Gerhart et al., 1989). Mitochondria disassociate from the yolk mass and are driven to aggregate into larger islands by cortical contraction waves and fusion (Ressom and Dixon, 1988; Savage and Danilchik, 1993; Perez-Mongiovi et al., 1998). The oligochaete *Tubifex* (Shimizu, 1986) and the cheatognath (Carre et al., 2002) both exhibit notable examples of aggregation of mitochondria (pole plasm and germ granule, respectively) due to cytoplasmic swirling. Aggregation of the mitochondria is also a feature of early cleavage in ascidians (Fig. 2e; Roegiers et al., 1999) *Drosophila* (Mahowold, 1971), and nematode (Strome and Wood, 1983); and perhaps humans as well (Wilding et al., 2001b), although time-lapse data do not exist to confirm this. In *Xenopus*, a localization of mitochondria to the future dorsal side of the egg is evident (Yost et al., 1995). While the majority of the mitochondria in the egg undergo secondary localization to the vegetal hemisphere, after fertilization, the remainder moves to the future dorsal midline of the egg, in the marginal zone. Relocation is a direct consequence of cytoplasmic movements during the first cell cycle (Yost et al., 1995), in which cortical rotation and sperm aster growth generate a deep cytoplasmic swirl indicative of the orientation of the dorsal/ventral axis (Danilchik and Denegre, 1991; Brown et al., 1993; Denegre and Danilchik, 1993).

An asymmetric distribution of mitochondria in the egg is often maintained and leads to the differential segregation of

mitochondria among blastomeres (Figs. 2e, f), as for example occurs in sea urchin embryos (Coffman et al., 2004; see below). In *Xenopus*, the islands of mitochondria in the germ plasm of the vegetal cortex ingress along the early cleavage furrows, elevating the mitochondria and germ plasm into the interior of the embryo (Savage and Danilchik, 1993) where they finally become incorporated into presumptive primordial germ cells near the blastocoel floor. The positioning of mitochondria to the future dorsal side of the egg persists in the embryo so that the prospective dorsal blastomeres are enriched in mitochondria and subsequently the boundary of enriched blastomeres is coincident with the location of the upper blastopore lip (Yost et al., 1995). Dorsal patterning is also highly correlated with segregation of mitochondria in blastomeres of *Tubifex*. By the fourth cleavage, the mitochondria of the pole plasm are segregated to the D-cell and its descendents. The distribution of mitochondria to the D-cell occurs concurrently with one of the earliest events specifying dorsal–ventral polarity, as the D-cell and progeny are known by cell ablation studies to be critical for dorsal–ventral patterning (Shimizu, 1989). In the ascidian, localizations in the egg are also maintained in the embryo, resulting in posterior vegetal cells containing the majority of the myoplasmic mitochondria, and anterior vegetal blastomeres also being enriched. Both sets of cells develop into muscle (Roegiers et al., 1999). Currently, no predictive correlations are associated with asymmetry of mitochondrial localization in blastomeres of mammalian embryos with respect to axial patterning, even though the asymmetries can be distinct. In human 2-cell and 8-cell embryos, differences in the mitochondrial segregation between blastomeres during cleavage are obvious and the differential localization can be traced back to asymmetric peri-nuclear aggregation at the one-cell stage (Van Blerkom et al., 2000; Van Blerkom, 2004). In mouse and pig embryos asymmetries between early blastomeres are present but not as distinct (Fig. 2f; Sun et al., 2001; Acton et al., 2004) and in rhesus monkey embryos blastomeres appear to be homogenous in mitochondrial distribution (Squirrell et al., 2003). The inability to predict axial patterning based on asymmetric localization of mitochondria in mammalian blastomeres is in part a consequence of a lack of visual or molecular cues for axial patterning at the early stages of development (but see discussion below of early markers of embryonic–abembryonic polarity), as well as the current lack of viable culture conditions which support time-lapse analysis of these events.

Mitochondrial structure and functions in axial patterning

The use of mitochondrial specific vital dyes, new high-resolution light microscopy techniques (Gugel et al., 2004) and live cell imaging has created a new picture of mitochondria as dynamic, reticulated networks (reviewed in Yaffe, 1999). Changes in the structure and distribution of mitochondria, and mitochondrial activity, have been associated with axial patterning in early development (Van Blerkom et al., 2000; Coffman and Davidson, 2001; Wilding et al., 2001b; Coffman et al., 2004; Dumollard et al., 2006). In the sea urchin embryo, a causal role for mitochondria in axial patterning is supported by

experimental manipulations such as egg centrifugation (which displaces mitochondria toward the centrifugal pole; Fig. 1d) and microinjection of purified mitochondria (Lindahl, 1932; Pease, 1939; Coffman et al., 2004; reviewed below).

In the mammalian egg, mitochondria have been called immature because they have no inner membrane cristae and were considered to be low in activity (Stern et al., 1971). During development, the mitochondria mature, gradually elongating and forming cristae (reviewed in Van Blerkom, 2004; Schatten et al., 2005). Supporting this idea is the observation that O_2 consumption is low in the egg and early embryo and increases as mitochondria mature (Trimarchi et al., 2000), presumably as mitochondria acquire the capacity to generate ATP. However, it is now clear that mitochondria in the egg are active and synthesize ATP (Dumollard et al., 2004), and that active mitochondria play a crucial role in the maintaining low resting levels of Ca^{2+} and maintaining the Ca^{2+} oscillations at fertilization which initiate early development (reviewed in Dumollard et al., 2006). The "immature" structure of mitochondria in the egg may be a consequence of this secondary role of mitochondrial metabolism, and the close association of mitochondria with ER (Marchant et al., 2002; reviewed in Van Blerkom, 2004; Dumollard et al., 2006) may enable cross-talk of soluble metabolites and signals. A similar relationship exists between mitochondrial activity and fertilization initiated Ca^{2+} waves in ascidians (Dumollard et al., 2003; reviewed in Sardet et al., 2007). The role of mitochondria in calcium modulation is not unique to eggs and embryos, as it appears to be a requirement for establishing neuronal polarity also (Mattson and Partin, 1999).

Localizations have also been defined by discrete mitochondrial activity defined by analyzing the redox regulation of the oocyte and embryo (Dumollard et al., 2007b), or by visualizing high membrane potential with vital dyes and direct measurement of ATP (reviewed in Dumollard et al., 2007a). Mitochondria in the Balbiani body of *Xenopus* oocytes, whose location is the earliest indicator of the AV axis, have been characterized as highly active compared to mitochondria dispersed in the cytoplasm (Wilding et al., 2001a). In mouse and human oocytes and embryos, localized mitochondria with high membrane potential are also observed. Oocytes have an asymmetric subcortical localization of high membrane potential mitochondria, which persists during cleavage so that a subset of individual blastomeres contain asymmetrically localized mitochondria with high membrane potential (Wilding et al., 2001b; Van Blerkom et al., 2002; Acton et al., 2004). A similar analysis of differences in mitochondrial function relating to localization was made by directly measuring ATP levels from isolated blastomeres with localized mitochondria (Van Blerkom et al., 2000), demonstrating higher levels of ATP and presumably greater mitochondrial activity. Results from direct measurements of mitochondrial activity in live cells suggests that quantitation of mitochondria by DNA content and replication is not optimal because mitochondria exist as reticulated networks which undergo extensive movements and fusions and therefore have no discrete unit for assay. Quantitation of mitochondria should instead be at the level of activity, such as redox state and

Ca^{2+} modulation and nitric oxide production (Manser and Houghton, 2006).

Redox signaling

To have an impact on development, mitochondria (or the chemistry associated with their activity) must affect the structure and function of molecules that regulate gene expression, which is proximally controlled by the combinatorial interactions of transcriptional regulatory proteins. A growing number of such proteins are known to be redox-regulated (Sun and Oberley, 1996; Fig. 3), and the phenomenon may well be more rule than exception. Examples include basic leucine zipper (b-Zip) proteins such as AP-1 (Toone et al., 2001; Valko et al., 2005; Amoutzias et al., 2006), rel family proteins such as NF-κB (Clive and Greene, 1996; Glineur et al., 2000), the nuclear respiratory factors NRF-1 and NRF-2 (Martin et al., 1996; Piantadosi and Suliman, 2006), homeodomain proteins such as Cdx2 and TTF-1 (Suh et al., 1994; Arnone et al., 1995), the paired-box protein Pax-8 (Cao et al., 2002, 2005), Runx proteins (Akamatsu et al., 1997), Myb proteins (Myrset et al.,

1993), and bHLH-PAS transcription factors involved in regulating diurnal metabolic cycles in metazoans (Rutter et al., 2001). Redox regulation of transcription factor activity often occurs at the level of DNA binding, as a result of conformational changes induced by oxidation or reduction of reactive cysteine residues within the DNA binding domain. It can also occur via redox-induced conformational changes in dimerization domains, activation domains, or phosphorylation sites; through effects on nuclear import/export; and/or by affecting the assembly of co-activator complexes, as in the case of the OCA-S co-activator (GAPDH), whose recruitment to the histone H2B promoter via Oct-1 is stimulated by NAD+ and inhibited by NADH (Zheng et al., 2003). Finally, redox state can regulate protein stability, as exemplified most famously by the hypoxia inducible factor HIF-1α.

HIF-1α is a subunit of the heterodimeric HIF transcription factor, which activates a battery of genes involved in cellular response to hypoxia, including that encoding vascular endothelial growth factor (VEGF), a secreted signaling ligand that promotes angiogenesis (reviewed by Maxwell and Ratcliffe, 2002). Steady state levels of HIF-1α protein are normally low
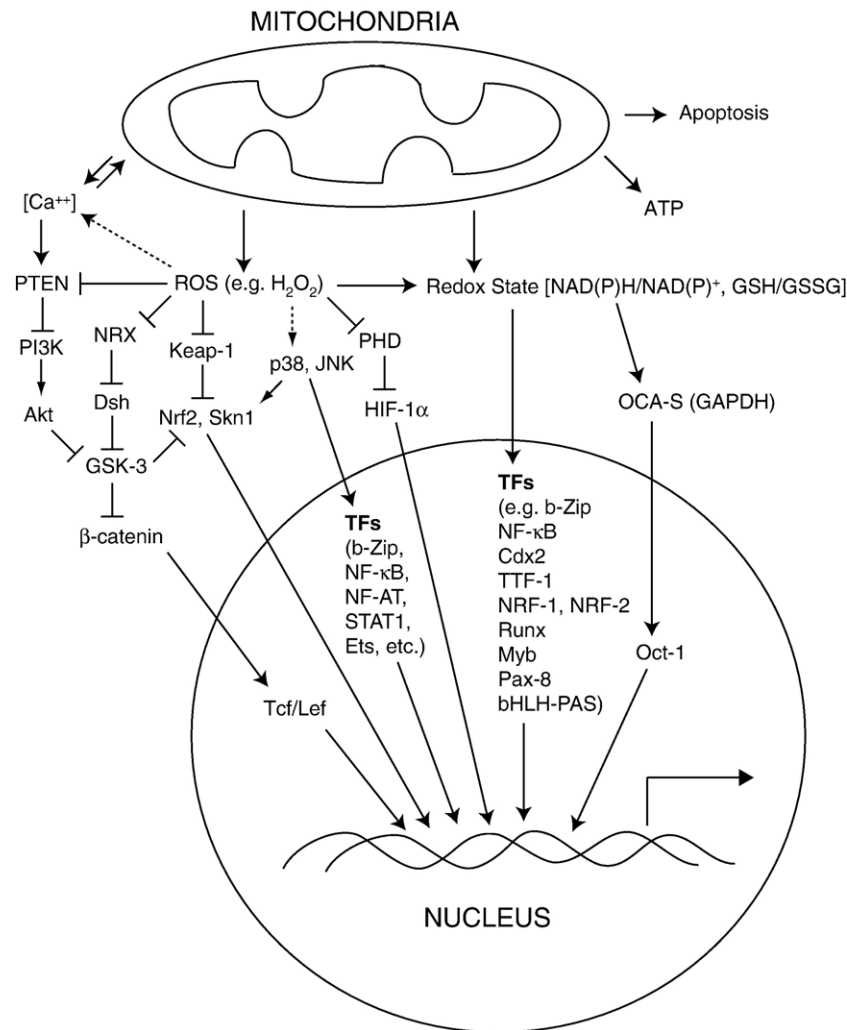

Fig. 3. Examples of mitochondrial redox signaling pathways that regulate gene activity. Large arrows depict unspecified regulatory effects; small arrows depict activation; barred lines depict inhibition or antagonism. For details and references, see text.

because it is targeted for proteolysis by proline hydroxylation followed by VHL-mediated ubiquitination (Ivan et al., 2001; Jaakkola et al., 2001). The prolyl hydroxylase (PHD) that targets HIF-1α for destruction is inactivated by low oxygen tension, allowing HIF-1α protein to accumulate. Whereas anoxia directly inactivates PHDs by removing a substrate required for their activity, hypoxia appears to do so indirectly by increasing mitochondrial production of ROS such as H_2O_2, which inhibits PHD activity through an as-yet poorly understood mechanism (Brunelle et al., 2005; Guzy et al., 2005; Mansfield et al., 2005).

Although HIF-1α is a key redox signaling factor, mitochondrial (Mt) H_2O_2 activates additional pathways (Fig. 3) that converge on genes important for angiogenesis and hence tissue oxygenation. For example, Mt H_2O_2 oxidizes and thereby reversibly inactivates PTEN (phosphatase and tensin homolog deleted from chromosome 10), a phosphatidylinositol 3,4,5-triphosphate $[PI(3,4,5)P_3]$ phosphatase that antagonizes phosphoinositide 3-kinase (PI3K) activity (Connor et al., 2005). Oxidative inhibition of PTEN up-regulates PI3K, which in turn activates Akt, a kinase whose activity stimulates VEGF production in addition to promoting cell survival and proliferation (reviewed in Brader and Eccles, 2004). Matrix metalloproteinases involved in angiogenic tissue remodeling are also induced by redox signals (Nelson and Melendez, 2004); for example the matrix metalloproteinase-1 (MMP-1) responds to Ets and AP-1 transcription factors that are in turn activated by Mt-H_2O_2-stimulated jun N-terminal kinase (JNK) and extracellular regulated kinase (ERK) activity (Wenk et al., 1999; Nelson et al., 2006).

ROS, particularly H_2O_2, generated either by mitochondria or intracellular oxidases have long been known to stimulate mitogen activated protein kinase (MAPK) signaling via the ERK, JNK and p38 MAPK (p38) pathways (e.g., Kulisz et al., 2002; Torres and Forman, 2003; Alonso et al., 2004; Fig. 3). ERKs generally regulate cell proliferation and differentiation in response to extracellular signals, whereas JNKs and p38 respond to oxidative and other types of intracellular stress (Torres and Forman, 2003). As p38 is particularly germane to the present discussion, we use it as an illustrative example. As with all of the MAPKs, the activity of p38 is regulated by its phosphorylation state: when phosphorylated on both threonine and tyrosine residues within the conserved tripeptide motif TxY, it accumulates in the nucleus and phosphorylates (and thereby activates) a number of transcription factors, including members of the b-Zip family. When de-phosphorylated, p38 is inactivated and clears from the nucleus. The phosphorylation state of p38 is controlled by a balance between the activities of upstream kinases and phosphatases, and it is likely that the redox-responsiveness of p38 is at least in part a function of one or more phosphatases that are inactivated by ROS (Robinson et al., 1999).

As is the case with PTEN, tyrosine and dual-specificity phosphatases are inactivated by the oxidation of a reactive cysteine residue near their catalytic site (den Hertog et al., 2005), and serine–threonine metallophosphatases are also known to be inhibited by oxidation (Namgaladze et al., 2002; Rao and Clayton, 2002). Since phosphatases have pleiotropic

substrate specificity, redox-regulated phosphatase activity affects many different signaling pathways and kinases, which include the calcium-calmodulin-dependent (CaM) kinases (Howe et al., 2004) in addition to the MAPKs and PI3K discussed above. It is therefore not unreasonable to suggest that redox regulation of signal transduction is a fundamental property of animal life (see Fig. 3 for summary of pathways discussed here). What might constitute the evolutionary antecedents of such regulation?

One likely possibility is that redox signaling descends from defense mechanisms that evolved to protect eukaryotic cells from ROS produced by mitochondria or environmental toxicants such as heavy metals. One line of such defense is represented by the so-called "Phase II" detoxification system, a battery of antioxidant genes that are coordinately regulated by the cap-n-collar (CNC)–b-Zip protein Nrf2 (NF-E2-related factor 2, unrelated to the nuclear respiratory factors NRF-1 and NRF-2) via a conserved cis-regulatory anti-oxidant response element (ARE; Lee and Johnson, 2004; Numazawa and Yoshida, 2004). Nrf2 is a redox-regulated transcription factor: under normal conditions it is retained in the cytoplasm through its association with the kelch domain protein Keap1, whereas in response to oxidative stress, it dissociates from Keap1, is stabilized, and accumulates in the nucleus to activate genes encoding enzymatic antioxidants such as glutathione S-transferase (Numazawa and Yoshida, 2004; Fig. 3). A similar (and probably homologous) b-Zip activated anti-oxidant defense network is found in yeast (Toone et al., 2001; Rodrigues-Pousada et al., 2005), suggesting that the system was assembled in eukaryotes prior to the evolution of metazoans.

In this context, the C. elegans regulatory gene skn-1 is particularly intriguing. SKN-1 is a transcription factor that was discovered by virtue of its involvement in endomesoderm specification (Bowerman et al., 1992, 1993; Maduro et al., 2001). It also regulates the expression of Phase II detoxification genes, and while structurally divergent, SKN-1 may nonetheless be a homologue of the b-Zip genes that fulfill the same function in other organisms (An and Blackwell, 2003). skn-1 mutants have impaired response to oxidative stress and shortened life spans (An and Blackwell, 2003). The activity of SKN-1 is positively regulated by p38, which is required for its nuclear localization (Inoue et al., 2005), and negatively regulated by GSK-3, which prevents its accumulation in the nucleus (An et al., 2005; Fig. 3). Human GSK-3β similarly inhibits Nrf2 nuclear localization and the attendant oxidative stress response (Salazar et al., 2006), suggesting that negative regulation of oxidative stress response may be an ancient function of this enzyme.

The fact that Phase II detoxification gene battery predates the evolution of Metazoa suggests that developmental pathways involved in endomesoderm specification may have been co-opted from primordial oxidative stress responses that evolved in our unicellular eukaryotic ancestors (An and Blackwell, 2003). Such a possibility is also consistent with the recent discovery that HIF-1-mediated hypoxia response pathway is involved in mesoderm specification in mice (Ramirez-Bergeron et al., 2004). Moreover, the β-catenin signaling pathway, a primitive regulator of endomesoderm specification (Wikramanayake et

al., 2003) that is antagonized by GSK-3β, is itself redox regulated. As noted above, oxidative inactivation of PTEN increases the activity of Akt, which phosphorylates and thereby inactivates GSK-3β (Connor et al., 2005), leading to increased β-catenin levels, activation of VEGF signaling, and consequent promotion of angiogenesis in endothelial cells (Skurk et al., 2005). Moreover, it was recently shown that disheveled (Dsh), a positive regulator of the β-catenin pathway, binds and is inhibited by nucleoredoxin (NRX, a thioredoxin family member), and this interaction is inhibited by oxidation of NRX, leading to an up-regulation of β-catenin signaling (Funato et al., 2006; Fig. 3). The connection between oxidative stress responses and endomeso-derm specification has some interesting implications for axis specification during the primordial evolution of Metazoa, as discussed in the final section of this review.

Mitochondria, redox signaling, and axis specification in sea urchin embryos

As might be surmised from the foregoing, there is abundant evidence indicating that (1) anisotropic distribution of mito-chondria is commonly found in embryos from across the phylogenetic spectrum, (2) redox signaling is often used to regulate gene expression, and (3) redox-signaling pathways have apparently been co-opted for endomesoderm specification in nematodes and probably other organisms as well. However, very little is known about how (or for that matter whether) these phenomena combine to contribute to spatial patterning in developing embryos. Currently, one of the best-positioned organisms for filling this gap is the sea urchin embryo, in part because its highly regulative mode of development is far less dependent on maternal localization of molecular determinants than is the development of other model organisms such as *C. elegans*, *Drosophila*, or even *Xenopus*, making it more plastic and responsive to environmental signaling (Blackstone and Bridge, 2005; Coffman, 2006). C. M. Child showed that both the primary (AV) and secondary (oral–aboral or OA) axes of the sea urchin embryo manifest metabolic gradients (Fig. 1), with the animal and oral poles of the early blastula displaying the highest levels of oxidative activity (Child, 1941). This work was extended by Gerhard Czihak, who demonstrated that these gradients are present as early as the 8-cell stage and attributable to the mito-chondrial enzyme cytochrome oxidase (Czihak, 1963). Previous experiments performed by Daniel Pease, who entrained OA pola-rity in sand dollar embryos by exposing them to steep concen-tration gradients of respiratory inhibitors (Pease, 1941, 1942a,b), suggest that the redox asymmetry observed by Czihak might play a role in specification of the OA axis. This possibility was corroborated by the recent demonstration that a mitochondrial respiratory gradient imposed by clustering of embryos tends to entrain both OA polarity and the activity of the P3A2 transcription factor (a NRF-1 homologue that spatially regulates gene expression along the OA axis; Coffman and Davidson, 2001), and that culturing embryos under a coverslip to limit oxygen suppresses specification of oral ectoderm (Coffman et al., 2004).

It was recently shown that mitochondria are asymmetrically distributed in unfertilized eggs of *Strongylocentrotus purpur-*

atus (Coffman et al., 2004). The polarity of this anisotropic distribution does not change significantly in the zygote, leading to an unequal apportioning of mitochondria to the blastomeres. The maternal mitochondrial asymmetry correlates with OA polarity, with the blastomeres inheriting the highest density of mitochondria tending to give rise to the oral pole of the embryo (Coffman et al., 2004). Importantly, the correlation between mitochondrial distribution and OA polarity holds following redistribution of mitochondria, either by centrifugal stratifica-tion of eggs, or by direct injection of purified mitochondria into zygotes (Coffman et al., 2004). Thus, specification of OA polarity appears to be entrained at least in part by a maternally specified anisotropy in mitochondrial distribution.

The key genetic event underlying ectodermal cell fate specification in normal sea urchin embryos is the localized zygotic expression of *nodal* in the prospective oral ectoderm at blastula stage, which establishes a signaling center that suffices to organize the OA axis by a classic reaction–diffusion mechanism involving Nodal and its downstream target and inhibitor Lefty/Antiven (Duboc et al., 2004). Hypoxia appears to radialize embryos by suppressing *nodal* expression (Coff-man et al., 2004), suggesting that *nodal* expression is redox regulated. One hypothesis to account for such regulation is that the *cis*-regulatory system that controls *nodal* transcription contains target sites for redox-sensitive transcription factors. A recent *cis*-regulatory analysis reveals that in *S. purpuratus nodal* is initially activated *via* consensus target sequences for b-Zip transcription factors (Nam et al., 2007), which as noted above are known to be redox regulated. A second, comple-mentary hypothesis is that *nodal* is responsive to transcription factors that are post-translationally modified by redox-sensitive signaling pathways. For reasons alluded to above, a particularly intriguing candidate for the latter is the p38 MAPK pathway, the activity of which is required for *nodal* activity and hence oral ectoderm specification (Bradham and McClay, 2005). In the sea urchin *Lytechinus variegatus*, p38 is transiently inactivated in the prospective aboral ectoderm at late blastula stage, and remains active in the prospective oral ectoderm (Bradham and McClay, 2005). As discussed above, p38 is activated by mitochondrial ROS such as H_2O_2, at least in part via oxidative inactivation of phosphatases (Torres and Forman, 2003). Thus, it is possible that the transient inactivation of p38 in the prospective aboral ectoderm is mediated by a globally distributed phosphatase that is held inactive by high levels of mitochondrial ROS in the prospective oral ectoderm.

A third hypothesis, not mutually exclusive of the first two but more consonant with Child's theory, is that the rate of *nodal* activity is limited by the overall metabolic rate of the cell. Cells with a higher density of mitochondria might be expected to have a higher rate of oxidative phosphorylation, which might in turn augment the rate of processes associated with growth and cell division. Indeed, protein synthesis is a particularly costly pro-cess in terms of energy consumption (Pace and Manahan, 2006), and hence its rate may be limited by differences in the rate of ATP production. Thus, the rate of *nodal* transcription may be limited initially by a transcription factor whose translation from maternal mRNA occurs at a higher rate in

mitochondria-rich cells, leading to a more rapid accumulation to the threshold concentration necessary for activity. Predictions of this hypothesis are that the early embryo should manifest an asymmetric rate of protein synthesis that is isomorphic with the asymmetries in mitochondrial distribution and *nodal* expression, and that a number of zygotically activated genes should display an asymmetric expression pattern that correlates with the mitochondrial asymmetry.

Testing each of these hypotheses will further illuminate the potential role played by mitochondria in specifying the secondary axis of the sea urchin embryo. Toward that end, some obvious experimental approaches include: (1) *cis*-regulatory analysis to determine whether *nodal* is activated by redox-responsive transcription factors (as appears to be the case; Nam et al., 2007); (2) targeting enzymatic or pharmacological anti-oxidants to mitochondria to test whether mitochondrial ROS are required for OA axis specification; (3) identifying a phosphatase involved in the transient inactivation of p38 in prospective aboral ectoderm, and determining whether it is redox regulated; and (4) determining whether the mitochondrial gradient in the early embryo correlates with spatially anisotropic rates of protein synthesis and gene expression.

Speculations and future directions

Prior to the evolution of stereotypical, maternally programmed embryogenesis, axis specification in early metazoans must have relied on spatial information generated stochastically by cellular activity and interactions. One of the earliest symmetry breaking events in primitive metazoans was likely the formation of multilayered balls of cells having an inside–outside axis, a process that in aerobic organisms invariably creates an oxygen gradient. It is possible that the regulatory pathways held in common between endomesoderm specification and oxidative stress responses are rooted in this primordial anisotropy, which would likely have activated redox-sensitive stress pathways that the first metazoans inherited from their unicellular eukaryotic ancestors. Maternal localization of regulatory molecules that were components of what was originally a redox-signaling pathway would then represent a subsequent evolutionary innovation that rigidified the ontogenetic program that segregates ectoderm and endomesoderm, ultimately becoming the principal (and obligate) mechanism underlying this specification event in many (most?) animals. Another spontaneous (unprogrammed) asymmetry that might have arisen in our earliest multicellular ancestors was an unequal distribution of mitochondria among dividing cells, leading to asymmetric activation of redox signaling and consequent specification of an axis, much as appears to occur in sea urchin embryos today. These considerations lead us to propose that mitochondria and redox signaling play an evolutionarily ancient and fundamental role in embryonic axis specification.

The following questions are thus raised: how widespread is axis specification through redox asymmetry among modern phyla? Is it an exception or the rule? Are there any animals other than echinoderms wherein mitochondria might play a leading role during embryogenesis?

Most work on developmental axis specification has been carried out in organisms that undergo a relatively "determinative" type of embryogenesis that is highly dependent upon localization of regulatory molecules: fruit flies, nematodes, ascidians and even frogs. If determinative development is indeed an evolutionarily derived specialization, then the best place to look for axis specification by redox asymmetry would be in embryos that are more regulative. Other than echinoderms, vertebrates (especially mammals) have the most regulative embryos. There is now substantial (albeit still somewhat controversial) evidence that the earliest observable axis of the mouse embryo – the embryonic–abembryonic (EA) axis – is at least tentatively specified in the early zygote, by an as yet poorly understood mechanism (Gardner, 2001; Piotrowska et al., 2001; Zernicka-Goetz, 2002, 2006; but see counterarguments of Motosugi et al., 2005; Kurotaki et al., 2007). The EA axis of the embryo appears to be predicted by an asynchronous second cleavage, with the early cleaving blastomere marking the embryonic pole (Piotrowska et al., 2001). While mitochondria are asymmetrically distributed in the cleaving mouse embryo (Fig. 2f; James Denegre and Jacquelyn Masse, unpublished data), it is not known what role (if any) they play in the asynchronous second cleavage or specification of the EA axis. The possibility that mitochondria are functionally part of this process should provide an interesting avenue for future research.

Finally, an important issue not given sufficient attention here due to space constraints is the relationship between mitochondria, redox signaling, and calcium. Mitochondria sequester calcium and interact with the endoplasmic reticulum (Rutter and Rizzuto, 2000) and are known to act as calcium buffers (Boitier et al., 1999; Parekh, 2003; Jacobson and Duchen, 2004; El Idrissi, 2006). Calcium uptake by mitochondria also regulates their metabolic activity and ROS production (Jacobson and Duchen, 2004). On the other hand, calcium channels and transporters are often redox regulated (Zima and Blatter, 2006), and calcium plays well-known roles in regulating many of the redox-signaling pathways discussed in this review. It therefore appears that redox signaling is reciprocally linked to calcium signaling, a fundamental connection that merits scrutiny in the context of animal development.

Acknowledgments

References

Acton, B.M., Jurisicova, A., Jurisica, I., Casper, R.F., 2004. Alterations in mitochondrial membrane potential during preimplantation stages of mouse and human embryo development. Mol. Hum. Reprod. 10, 23–32.

Akamatsu, Y., Ohno, T., Hirota, K., Kagoshima, H., Yodoi, J., Shigesada, K., 1997. Redox regulation of the DNA binding activity in transcription factor PEBP2. The roles of two conserved cysteine residues. J. Biol. Chem. 272, 14497–14500.

Alonso, M., Melani, M., Converso, D., Jaitovich, A., Paz, C., Carreras, M.C., Medina, J.H., Poderoso, J.J., 2004. Mitochondrial extracellular signal-regulated kinases 1/2 (ERK1/2) are modulated during brain development. J. Neurochem. 89, 248–256.

Amoutzias, G.D., Bornberg-Bauer, E., Oliver, S.G., Robertson, D.L., 2006. Reduction/oxidation-phosphorylation control of DNA binding in the bZIP dimerization network. BMC Genomics 7, 107.

An, J.H., Blackwell, T.K., 2003. SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response. Genes Dev. 17, 1882–1893.

An, J.H., Vranas, K., Lucke, M., Inoue, H., Hisamoto, N., Matsumoto, K., Blackwell, T.K., 2005. Regulation of the *Caenorhabditis elegans* oxidative stress defense protein SKN-1 by glycogen synthase kinase-3. Proc. Natl. Acad. Sci. U. S. A. 102, 16275–16280.

Arnone, M.I., Zannini, M., Di Lauro, R., 1995. The DNA binding activity and the dimerization ability of the thyroid transcription factor I are redox regulated. J. Biol. Chem. 270, 12048–12055.

Bardsley, A., McDonald, K., Boswell, R.E., 1993. Distribution of tudor protein in the *Drosophila* embryo suggests separation of functions based on site of localization. Development 119, 207–219.

Bement, W.M., Capco, D.G., 1990. Transformation of the amphibian oocyte into the egg: structural and biochemical events. J. Electron. Microsc. Tech. 16, 202–234.

Blackler, A.W., 1958. Contribution to the study of germ-cells in the anura. J. Embryol. Exp. Morphol. 6, 491–503.

Blackstone, N.W., 2000. Redox control and the evolution of multicellularity. BioEssays 22, 947–953.

Blackstone, N.W., 2003. Redox signaling in the growth and development of colonial hydroids. J. Exp. Biol. 206, 651–658.

Blackstone, N.W., 2006. Charles Manning Child (1869–1954): the past, present, and future of metabolic signaling. J. Exp. Zool. B Mol. Dev. Evol. 306, 1–7.

Blackstone, N.W., Bridge, D.M., 2005. Model systems for environmental signaling. Integr. Comp. Biol. 45, 605–614.

Blackstone, N.W., Bivins, M.J., Cherry, K.S., Fletcher, R.E., Geddes, G.C., 2005. Redox signaling in colonial hydroids: many pathways for peroxide. J. Exp. Biol. 208, 383–390.

Boitier, E., Rea, R., Duchen, M.R., 1999. Mitochondria exert a negative feedback on the propagation of intracellular Ca^{2+} waves in rat cortical astrocytes. J. Cell Biol. 145, 795–808.

Bowerman, B., Eaton, B.A., Priess, J.R., 1992. *skn-1*, a maternally expressed gene required to specify the fate of ventral blastomeres in the early *C. elegans* embryo. Cell 68, 1061–1075.

Bowerman, B., Draper, B.W., Mello, C.C., Priess, J.R., 1993. The maternal gene *skn-1* encodes a protein that is distributed unequally in early *C. elegans* embryos. Cell 74, 443–452.

Brader, S., Eccles, S.A., 2004. Phosphoinositide 3-kinase signalling pathways in tumor progression, invasion and angiogenesis. Tumori 90, 2–8.

Bradham, C.A., McClay, D.R., 2005. p38 MAPK is essential for secondary axis specification and patterning in sea urchin embryos. Development 133, 21–32.

Brown, E.E., Denegre, J.M., Danilchik, M.V., 1993. Deep cytoplasmic rearrangements in ventralized *Xenopus* embryos. Dev. Biol. 160, 148–156.

Brunelle, J.K., Bell, E.L., Quesada, N.M., Vercauteren, K., Tiranti, V., Zeviani, M., Scarpulla, R.C., Chandel, N.S., 2005. Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. Cell Metab. 1, 409–414.

Calarco, P.G., 1995. Polarization of mitochondria in the unfertilized mouse oocyte. Dev. Genet. 16, 36–43.

Cao, X., Kambe, F., Ohmori, S., Seo, H., 2002. Oxidoreductive modification of two cysteine residues in paired domain by Ref-1 regulates DNA-binding activity of Pax-8. Biochem. Biophys. Res. Commun. 297, 288–293.

Cao, X., Kambe, F., Lu, X., Kobayashi, N., Ohmori, S., Seo, H., 2005. Glutathionylation of two cysteine residues in paired domain regulates DNA binding activity of Pax-8. J. Biol. Chem. 280, 25901–25906.

Carre, D., Djediat, C., Sardet, C., 2002. Formation of a large Vasa-positive germ granule and its inheritance by germ cells in the enigmatic Chaetognaths. Development 129, 661–670.

Chang, P., Torres, J., Lewis, R.A., Mowry, K.L., Houliston, E., King, M.L., 2004. Localization of RNAs to the mitochondrial cloud in *Xenopus* oocytes through entrapment and association with endoplasmic reticulum. Mol. Biol. Cell 15, 4669–4681.

Child, C.M., 1941. Patterns and Problems of Development. University of Chicago Press, Chicago, IL.

Clive, D.R., Greene, J.J., 1996. Cooperation of protein disulfide isomerase and redox environment in the regulation of NF-kappaB and AP1 binding to DNA. Cell Biochem. Funct. 14, 49–55.

Coffman, J.A., 2006. Developmental ascendency: from bottom–up to top–down control. Biol. Theor. 1, 165–178.

Coffman, J.A., Davidson, E.H., 2001. Oral–aboral axis specification in the sea urchin embryo: I. Axis entrainment by respiratory asymmetry. Dev. Biol. 230, 18–28.

Coffman, J.A., McCarthy, J.J., Dickey-Sims, C., Robertson, A.J., 2004. Oral–aboral axis specification in the sea urchin embryo: II. Mitochondrial distribution and redox state contribute to establishing polarity in *Strongylocentrotus purpuratus*. Dev. Biol. 273, 160–171.

Conklin, E.G., 1905. The orientation and cell-lineage of the ascidian egg. J. Acad. Nat. Sci. Phila. 13, 5–119.

Conklin, E.G., 1931. The development of centrifuged eggs of ascidians. J. Exp. 60.

Connor, K.M., Subbaram, S., Regan, K.J., Nelson, K.K., Mazurkiewicz, J.E., Bartholomew, P.J., Aplin, A.E., Tai, Y.T., Aguirre-Ghiso, J., Flores, S.C., Melendez, J.A., 2005. Mitochondrial H_2O_2 regulates the angiogenic phenotype via PTEN oxidation. J. Biol. Chem. 280, 16916–16924.

Cox, R.T., Spradling, A.C., 2006. Milton controls the early acquisition of mitochondria by *Drosophila* oocytes. Development 133, 3371–3377.

Czihak, G., 1963. Entwicklungsphysiologische untersuchungen an echiniden (Verteilung und bedeutung der cytochromoxydase). Roux' Arch. Entwickl. mech 154, 272–292.

Danilchik, M.V., Denegre, J.M., 1991. Deep cytoplasmic rearrangements during early development in *Xenopus laevis*. Development 111, 845–856.

Davidson, E.H., 1986. Gene Activity in Early Development. Academic Press, San Diego.

Davidson, E.H. 2006. "The Regulatory Genome: Gene Regulatory Networks in Development and Evolution." Academic Press/Elsevier, San Diego.

Denegre, J.M., Danilchik, M.V., 1993. Deep cytoplasmic rearrangements in axis-respecified *Xenopus* embryos. Dev. Biol. 160, 157–164.

Denegre, J.M., Ludwig, E.R., Mowry, K.L., 1997. Localized maternal proteins in *Xenopus* revealed by subtractive immunization. Dev. Biol. 192, 446–454.

den Hertog, J., Groen, A., van der Wijk, T., 2005. Redox regulation of protein-tyrosine phosphatases. Arch. Biochem. Biophys. 434, 11–15.

Deshler, J.O., Highett, M.I., Schnapp, B.J., 1997. Localization of *Xenopus* Vg1 mRNA by Vera protein and the endoplasmic reticulum. Science 276, 1128–1131.

Duboc, V., Rottinger, E., Besnardeau, L., Lepage, T., 2004. Nodal and BMP2/4 signaling organizes the oral–aboral axis of the sea urchin embryo. Dev. Cell 6, 397–410.

Dumollard, R., Hammar, K., Porterfield, M., Smith, P.J., Cibert, C., Rouviere, C., Sardet, C., 2003. Mitochondrial respiration and Ca^{2+} waves are linked during fertilization and meiosis completion. Development 130, 683–692.

Dumollard, R., Marangos, P., Fitzharris, G., Swann, K., Duchen, M., Carroll, J., 2004. Sperm-triggered $[Ca^{2+}]$ oscillations and Ca^{2+} homeostasis in the mouse egg have an absolute requirement for mitochondrial ATP production. Development 131, 3057–3067.

Dumollard, R., Duchen, M., Sardet, C., 2006. Calcium signals and mitochondria at fertilisation. Semin. Cell Dev. Biol. 17, 314–323.

Dumollard, R., Duchen, M., Carroll, J., 2007a. The role of mitochondrial function in the oocyte and embryo. Curr. Top. Dev. Biol. 77, 21–49.

Dumollard, R., Ward, Z., Carroll, J., Duchen, M.R., 2007b. Regulation of redox metabolism in the mouse oocyte and embryo. Development 134, 455–465.

Eddy, E.M., 1975. Germ plasm and the differentiation of the germ cell line. Int. Rev. Cytol. 43, 229–280.

El Idrissi, A., 2006. Taurine increases mitochondrial buffering of calcium: role in neuroprotection. Amino Acids. doi:10.1007/s00726-006-0396-9.

Extavour, C.G., Akam, M., 2003. Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. Development 130, 5869–5884.

Funato, Y., Michiue, T., Asashima, M., Miki, H., 2006. The thioredoxin-related redox-regulating protein nucleoredoxin inhibits Wnt-beta-catenin signalling through *dishevelled*. Nat. Cell Biol. 8, 501–508.

Gardner, R.L., 2001. Specification of embryonic axes begins before cleavage in normal mouse development. Development 128, 839–847.

Gerhart, J., 1980. Mechanisms regulating pattern formation in the amphibian egg and early embryo. In: Goldgberger, R. (Ed.), Biological Regulation and Development, vol. 2. Plenum Press, New York, pp. 136–316.

Gerhart, J., Danilchik, M., Doniach, T., Roberts, S., Rowning, B., Stewart, R., 1989. Cortical rotation of the *Xenopus* egg: consequences for the anteroposterior pattern of embryonic dorsal development. Development 107, 37–51.

Glineur, C., Davioud-Charvet, E., Vandenbunder, B., 2000. The conserved redox-sensitive cysteine residue of the DNA-binding region in the c-Rel protein is involved in the regulation of the phosphorylation of the protein. Biochem. J. 352 (Pt. 2), 583–591.

Gugel, H., Bewersdorf, J., Jakobs, S., Engelhardt, J., Storz, R., Hell, S.W., 2004. Cooperative 4Pi excitation and detection yields sevenfold sharper optical sections in live-cell microscopy. Biophys. J. 87, 4146–4152.

Guraya, S.S., 1979. Recent advances in the morphology, cytochemistry, and function of Balbiani's vitelline body in animal oocytes. Int. Rev. Cytol. 59, 249–321.

Guzy, R.D., Hoyos, B., Robin, E., Chen, H., Liu, L., Mansfield, K.D., Simon, M.C., Hammerling, U., Schumacker, P.T., 2005. Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. Cell Metab. 1, 401–408.

Harvey, E.B., 1941. Vital staining of the centrifuged *Arbacia punctulata* egg. Biol. Bull. 81, 114–118.

Heasman, J., Quarmby, J., Wylie, C.C., 1984. The mitochondrial cloud of *Xenopus* oocytes: the source of germinal granule material. Dev. Biol. 105, 458–469.

Hertig, A.T., 1968. The primary human oocyte: some observations on the fine structure of Balbiani's vitelline body and the origin of the annulate lamellae. Am. J. Anat. 122, 107–137.

Hertig, A.T., Adams, E.C., 1967. Studies on the human oocyte and its follicle: I. Ultrastructural and histochemical observations on the primordial follicle stage. J. Cell Biol. 34, 647–675.

Hiiragi, T., Louvet-Vallee, S., Solter, D., Maro, B., 2006. Embryology: does prepatterning occur in the mouse egg? Nature 442, E3–E4 (discussion E4).

Houchmandzadeh, B., Wieschaus, E., Leibler, S., 2002. Establishment of developmental precision and proportions in the early *Drosophila* embryo. Nature 415, 798–802.

Howe, C.J., Lahair, M.M., McCubrey, J.A., Franklin, R.A., 2004. Redox regulation of the calcium/calmodulin-dependent protein kinases. J. Biol. Chem. 279, 44573–44581.

Illmensee, K., Mahowald, A.P., 1974. Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. Proc. Natl. Acad. Sci. U. S. A. 71, 1016–1020.

Illmensee, K., Mahowald, A.P., 1976. The autonomous function of germ plasm in a somatic region of the *Drosophila* egg. Exp. Cell Res. 97, 127–140.

Illmensee, K., Mahowald, A.P., Loomis, M.R., 1976. The ontogeny of germ plasm during oogenesis in *Drosophila*. Dev. Biol. 49, 40–65.

Inoue, H., Hisamoto, N., An, J.H., Oliveira, R.P., Nishida, E., Blackwell, T.K., Matsumoto, K., 2005. The *C. elegans* p38 MAPK pathway regulates nuclear localization of the transcription factor SKN-1 in oxidative stress response. Genes Dev. 19, 2278–2283.

Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J.M., Lane, W.S., Kaelin Jr., G., 2001. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. Science 292, 464–468.

Jaakkola, P., Mole, D.R., Tian, Y.M., Wilson, M.I., Gielbert, J., Gaskell, S.J., Kriegsheim, A., Hebestreit, H.F., Mukherji, M., Schofield, C.J., Maxwell, P.H., Pugh, C.W., Ratcliffe, P.J., 2001. Targeting of HIF-alpha to the von Hippel–Landau ubiquitylation complex by O_2-regulated prolyl hydroxylation. Science 292, 468–472.

Jacobson, J., Duchen, M.R., 2004. Interplay between mitochondria and cellular calcium signalling. Mol. Cell. Biochem. 256–257, 209–218.

Jaglarz, M.K., Nowak, Z., Bilinski, S.M., 2003. The Balbiani body and generation of early asymmetry in the oocyte of a tiger beetle. Differentiation 71, 142–151.

Jeffery, W.R., 1988. The role of cytoplasmic determinants in embryonic development. Dev. Biol. (N Y 1988) 5, 3–56.

Jeffery, W.R., Swalla, B.J., 1990. The myoplasm of ascidian eggs: a localized cytoskeletal domain with multiple roles in embryonic development. Semin. Cell Biol. 1, 373–381.

King, M.L., Messitt, T.J., Mowry, K.L., 2005. Putting RNAs in the right place at the right time: RNA localization in the frog oocyte. Biol. Cell 97, 19–33.

Kulisz, A., Chen, N., Chandel, N.S., Shao, Z., Schumacker, P.T., 2002. Mitochondrial ROS initiate phosphorylation of p38 MAP kinase during hypoxia in cardiomyocytes. Am. J. Physiol.: Lung Cell Mol. Physiol. 282, L1324–L1329.

Kumano, G., Nishida, H., 2007. Ascidian embryonic development: an emerging model system for the study of cell fate specification in chordates. Dev. Dyn. doi:10.1002/dvdy.21108.

Kurotaki, Y., Hatta, K., Nakao, K., Nabeshima, Y., Fujimori, T., 2007. Blastocyst axis is specified independently of early cell lineage but aligns with the ZP shape. Science 316, 719–723.

Lee, J.M., Johnson, J.A., 2004. An important role of Nrf2–ARE pathway in the cellular defense mechanism. J. Biochem. Mol. Biol. 37, 139–143.

Lindahl, P.E., 1932. Zur experimentellen analysis der determination der dorsoventralachse beim seeigelkeim: II. Versuche mit zentrifugierten eiern. Roux'Arch. Entwickl.mech. 127, 323–338.

Maduro, M.F., Meneghini, M.D., Bowerman, B., Broitman-Maduro, G., Rothman, J.H., 2001. Restriction of mesendoderm to a single blastomere by the combined action of SKN-1 and a GSK-3beta homolog is mediated by MED-1 and -2 in *C. elegans*. Mol. Cell 7, 475–485.

Mahowald, A.P., 1971a. Polar granules of *Drosophila*: 3. The continuity of polar granules during the life cycle of *Drosophila*. J. Exp. Zool. 176, 329–343.

Mahowald, A.P., 1971b. Polar granules of *Drosophila*: IV. Cytochemical studies showing loss of RNA from polar granules during early stages of embryogenesis. J. Exp. Zool. 176, 345–352.

Manser, R.C., Houghton, F.D., 2006. Ca^{2+}-linked upregulation and mitochondrial production of nitric oxide in the mouse preimplantation embryo. J. Cell Sci. 119, 2048–2055.

Mansfield, K.D., Guzy, R.D., Pan, Y., Young, R.M., Cash, T.P., Schumacker, P.T., Simon, M.C., 2005. Mitochondrial dysfunction resulting from loss of cytochrome *c* impairs cellular oxygen sensing and hypoxic HIF-alpha activation. Cell Metab. 1, 393–399.

Marchant, J.S., Ramos, V., Parker, I., 2002. Structural and functional relationships between Ca^{2+} puffs and mitochondria in *Xenopus* oocytes. Am. J. Physiol.: Cell Physiol. 282, C186–C1374.

Martin, M.E., Chinenov, Y., Yu, M., Schmidt, T.K., Yang, X.Y., 1996. Redox regulation of GA-binding protein-alpha DNA binding activity. J. Biol. Chem. 271, 25617–25623.

Mattson, M.P., Partin, J., 1999. Evidence for mitochondrial control of neuronal polarity. J. Neurosci. Res. 56, 8–20.

Maxwell, P.H., Ratcliffe, P.J., 2002. Oxygen sensors and angiogenesis. Semin. Cell Dev. Biol. 13, 29–37.

Mignotte, F., Tourte, M., Mounolou, J.C., 1987. Segregation of mitochondria in the cytoplasm of *Xenopus* vitellogenic oocytes. Biol. Cell 60, 97–102.

Morgan, T.H., Lyon, E.P., 1907. The relation of the substances of the egg, separated by strong centrifugal force, to the location of the embryo. Arch. F. Entwickl.mech. 24, 147.

Morgan, T.H., Spooner, G.B., 1909. The polarity of the centrifuged egg. Arch. F. Entwickl.mech. 28, 104.

Motosugi, N., Bauer, T., Polanski, Z., Solter, D., Hiiragi, T., 2005. Polarity of the mouse embryo is established at blastocyst and is not prepatterned. Genes Dev. 19, 1081–1092.

Mowry, K.L., Cote, C.A., 1999. RNA sorting in *Xenopus* oocytes and embryos. FASEB J. 13, 435–445.

Myrset, A.H., Bostad, A., Jamin, N., Lirsac, P.N., Toma, F., Gabrielsen, O.S., 1993. DNA and redox state induced conformational changes in the DNA-binding domain of the Myb oncoprotein. EMBO J. 12, 4625–4633.

Nam, J., Su, Y.-H., Lee, P.Y., Robertson, A.J., Coffman, A.J., Davidson, E.H., 2007. *Cis*-regulatory control of the *nodal* gene, initiator of the sea urchin oral ectoderm gene network. Dev. Biol. 306, 860–869.

Namgaladze, D., Hofer, H.W., Ullrich, V., 2002. Redox control of calcineurin by targeting the binuclear Fe(2+)–Zn(2+) center at the enzyme active site. J. Biol. Chem. 277, 5962–5969.

Nelson, K.K., Melendez, J.A., 2004. Mitochondrial redox control of matrix metalloproteinases. Free Radical Biol. Med. 37, 768–784.

Nelson, K.K., Subbaram, S., Connor, K.M., Dasgupta, J., Ha, X.F., Meng, T.C., Tonks, N.K., Melendez, J.A., 2006. Redox-dependent matrix metalloproteinase-1 expression is regulated by JNK through Ets and AP-1 promoter motifs. J. Biol. Chem. 281, 14100–14110.

Nijhout, H.F., 2002. The nature of robustness in development. BioEssays 24, 553–563.

Nishida, H., 2005. Specification of embryonic axis and mosaic development in ascidians. Dev. Dyn. 233, 1177–1193.

Nishida, H., Sawada, K., 2001. *macho-1* encodes a localized mRNA in ascidian eggs that specifies muscle fate during embryogenesis. Nature 409, 724–729.

Numazawa, S., Yoshida, T., 2004. Nrf2-dependent gene expressions: a molecular toxicological aspect. J. Toxicol. Sci. 29, 81–89.

Pace, D.A., Manahan, D.T., 2006. Fixed metabolic costs for highly variable rates of protein synthesis in sea urchin embryos and larvae. J. Exp. Biol. 209, 158–170.

Parekh, A.B., 2003. Mitochondrial regulation of intracellular Ca^{2+} signaling: more than just simple Ca^{2+} buffers. News Physiol. Sci. 18, 252–256.

Pease, D.C., 1939. An analysis of the factors of bilateral determination in centrifuged echinoderm embryos. J. Exp. Zool. 80, 225–247.

Pease, D.C., 1941. Echinoderm bilateral determination in chemical concentration gradients: I. The effects of cyanide, ferricyanide, iodoacetate, picrate, dinitrophenol, urethane, iodine, malonate, etc. J. Exp. Zool. 86, 381–404.

Pease, D.C., 1942a. Echinoderm bilateral determination in chemical concentration gradients: II. The effects of azide, pilocarpine, pyocyanine, diamine, cysteine, glutathione, and lithium. J. Exp. Zool. 89, 329–345.

Pease, D.C., 1942b. Echinoderm bilateral determination in chemical concentration gradients: III. The effects of carbon monoxide and other gases. J. Exp. Zool. 89, 347–356.

Pepling, M.E., Wilhelm, J.E., O'Hara A, L., Gephardt, G.W., Spradling, A.C., 2007. Mouse oocytes within germ cell cysts and primordial follicles contain a Balbiani body. Proc. Natl. Acad. Sci. U. S. A. 104, 187–192.

Perez-Mongiovi, D., Chang, P., Houliston, E., 1998. A propagated wave of MPF activation accompanies surface contraction waves at first mitosis in *Xenopus*. J. Cell Sci. 111 (Pt. 3), 385–393.

Piantadosi, C.A., Suliman, H.B., 2006. Mitochondrial transcription factor A induction by redox activation of nuclear respiratory factor 1. J. Biol. Chem. 281, 324–333.

Piotrowska, K., Wianny, F., Pedersen, R.A., Zernicka-Goetz, M., 2001. Blastomeres arising from the first cleavage division have distinguishable fates in normal mouse development. Development 128, 3739–3748.

Prodon, F., Dru, P., Roegiers, F., Sardet, C., 2005. Polarity of the ascidian egg cortex and relocalization of cER and mRNAs in the early embryo. J. Cell Sci. 118, 2393–2404.

Prodon, F., Chenevert, J., Sardet, C., 2006. Establishment of animal–vegetal polarity during maturation in ascidian oocytes. Dev. Biol. 290, 297–311.

Prodon, F., Yamada, L., Shirae-Kurabayashi, M., Nakamura, Y., Sasakura, Y., 2007. Postplasmic/PEM RNAs: a class of localized maternal mRNAs with multiple roles in cell polarity and development in ascidian embryos. Dev. Dyn. doi:10.1002/dvdy.21109.

Ramirez-Bergeron, D.L., Runge, A., Dahl, K.D., Fehling, H.J., Keller, G., Simon, M.C., 2004. Hypoxia affects mesoderm and enhances hemangioblast specification during early development. Development 131, 4623–4634.

Rao, R.K., Clayton, L.W., 2002. Regulation of protein phosphatase 2A by hydrogen peroxide and glutathionylation. Biochem. Biophys. Res. Commun. 293, 610–616.

Ressom, R.E., Dixon, K.E., 1988. Relocation and reorganization of germ plasm in *Xenopus* embryos after fertilization. Development 103, 507–518.

Reverbari, G., 1956. The mitochondrial pattern in the development of the ascidian egg. Experientia 41, 55–56.

Robinson, K.A., Stewart, C.A., Pye, Q.N., Nguyen, X., Kenney, L., Salzman, S., Floyd, R.A., Hensley, K., 1999. Redox-sensitive protein phosphatase activity regulates the phosphorylation state of p38 protein kinase in primary astrocyte culture. J. Neurosci. Res. 55, 724–732.

Rodrigues-Pousada, C., Nevitt, T., Menezes, R., 2005. The yeast stress response. Role of the Yap family of b-ZIP transcription factors. The PABMB Lecture delivered on 30 June 2004 at the 29th FEBS Congress in Warsaw. FEBS J. 272, 2639–2647.

Roegiers, F., McDougall, A., Sardet, C., 1995. The sperm entry point defines the orientation of the calcium-induced contraction wave that directs the first phase of cytoplasmic reorganization in the ascidian egg. Development 121, 3457–3466.

Roegiers, F., Djediat, C., Dumollard, R., Rouviere, C., Sardet, C., 1999. Phases of cytoplasmic and cortical reorganizations of the ascidian zygote between fertilization and first division. Development 126, 3101–3117.

Rutter, G.A., Rizzuto, R., 2000. Regulation of mitochondrial metabolism by ER Ca^{2+} release: an intimate connection. Trends Biochem. Sci. 25, 215–221.

Rutter, J., Reick, M., Wu, L.C., McKnight, S.L., 2001. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. Science 293, 510–514.

Salazar, M., Rojo, A.I., Velasco, D., de Sagarra, R.M., Cuadrado, A., 2006. Glycogen synthase kinase-3beta inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. J. Biol. Chem. 281, 14841–14851.

Sardet, C., Nishida, H., Prodon, F., Sawada, K., 2003. Maternal mRNAs of *PEM* and *macho 1*, the ascidian muscle determinant, associate and move with a rough endoplasmic reticulum network in the egg cortex. Development 130, 5839–5849.

Sardet, C., Dru, P., Prodon, F., 2005. Maternal determinants and mRNAs in the cortex of ascidian oocytes, zygotes and embryos. Biol. Cell 97, 35–49.

Sardet, C., Paix, A., Prodon, F., Dru, P., Chenevert, J., 2007. From oocyte to 16-cell stage: cytoplasmic and cortical reorganizations that pattern the ascidian embryo. Dev. Dyn. doi:10.1002/dvdy.21136.

Satoh, N., Araki, I., Satou, Y., 1996. An intrinsic genetic program for autonomous differentiation of muscle cells in the ascidian embryo. Proc. Natl. Acad. Sci. U. S. A. 93, 9315–9321.

Savage, R.M., Danilchik, M.V., 1993. Dynamics of germ plasm localization and its inhibition by ultraviolet irradiation in early cleavage *Xenopus* embryos. Dev. Biol. 157, 371–382.

Schatten, H., Prather, R.S., Sun, Q.Y., 2005. The significance of mitochondria for embryo development in cloned farm animals. Mitochondrion 5, 303–321.

Shimizu, T., 1986. Bipolar segregation of mitochondria, actin network, and surface in the *Tubifex* egg: role of cortical polarity. Dev. Biol. 116, 241–251.

Shimizu, T., 1989. Asymmetric segregation and polarized redistribution of pole plasm during early cleavages in the *Tubifex* embryo: role of actin networks and mitotic apparatus. Dev. Growth Differ. 31, 283–297.

Skurk, C., Maatz, H., Rocnik, E., Bialik, A., Force, T., Walsh, K., 2005. Glycogen-synthase kinase3beta/beta-catenin axis promotes angiogenesis through activation of vascular endothelial growth factor signaling in endothelial cells. Circ. Res. 96, 308–318.

Smith, L.D., 1966. The role of a "germinal plasm" in the formation of primordial germ cells in *Rana pipiens*. Dev. Biol. 14, 330–347.

Smith, L.D., Williams, M.A., 1975. Germinal plasm and determination of the primordial germ cells. Symp. Soc. Dev. 3–24.

Smith, J., Ladi, E., Mayer-Proschel, M., Noble, M., 2000. Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell. Proc. Natl. Acad. Sci. U. S. A. 97, 10032–10037.

Solnica-Krezel, L., 2003. Vertebrate development: taming the nodal waves. Curr. Biol. 13, R7–R9.

Speksnijder, J.E., 1992. The repetitive calcium waves in the fertilized ascidian egg are initiated near the vegetal pole by a cortical pacemaker. Dev. Biol. 153, 259–271.

Squirrell, J.M., Schramm, R.D., Paprocki, A.M., Wokosin, D.L., Bavister, B.D., 2003. Imaging mitochondrial organization in living primate oocytes and embryos using multiphoton microscopy. Microsc. Microanal. 9, 190–201.

Stern, S., Biggers, J.D., Anderson, E., 1971. Mitochondria and early development of the mouse. J. Exp. Zool. 176, 179–191.

Strome, S., Wood, W.B., 1982. Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U. S. A. 79, 1558–1562.

Strome, S., Wood, W.B., 1983. Generation of asymmetry and segregation of germ-line granules in early *C. elegans* embryos. Cell 35, 15–25.

Suh, E., Chen, L., Taylor, J., Traber, P.G., 1994. A homeodomain protein related to caudal regulates intestine-specific gene transcription. Mol. Cell. Biol. 14, 7340–7351.

Sun, Y., Oberley, L.W., 1996. Redox regulation of transcriptional activators. Free Radical Biol. Med. 21, 335–348.

Sun, Q.Y., Wu, G.M., Lai, L., Park, K.W., Cabot, R., Cheong, H.T., Day, B.N., Prather, R.S., Schatten, H., 2001. Translocation of active mitochondria during pig oocyte maturation, fertilization and early embryo development in vitro. Reproduction 122, 155–163.

Terasaki, M., Runft, L.L., Hand, A.R., 2001. Changes in organization of the endoplasmic reticulum during Xenopus oocyte maturation and activation. Mol. Biol. Cell 12, 1103–1116.

Toone, W.M., Morgan, B.A., Jones, N., 2001. Redox control of AP-1-like factors in yeast and beyond. Oncogene 20, 2336–2346.

Torres, M., Forman, H.J., 2003. Redox signaling and the MAP kinase pathways. BioFactors 17, 287–296.

Tourte, M., Mignotte, F., Mounolou, J.C., 1984. Heterogeneous distribution and replication activity of mitochondria in Xenopus laevis oocytes. Eur. J. Cell Biol. 34, 171–178.

Trimarchi, J.R., Liu, L., Porterfield, D.M., Smith, P.J., Keefe, D.L., 2000. Oxidative phosphorylation-dependent and -independent oxygen consumption by individual preimplantation mouse embryos. Biol. Reprod. 62, 1866–1874.

Turing, A.M., 1952. The chemical basis of morphogenesis. Bull. Math. Biol. 52, 153–197.

Valko, M., Morris, H., Cronin, M.T., 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem. 12, 1161–1208.

Van Blerkom, J., 1991. Microtubule mediation of cytoplasmic and nuclear maturation during the early stages of resumed meiosis in cultured mouse oocytes. Proc. Natl. Acad. Sci. U. S. A. 88, 5031–5035.

Van Blerkom, J., 2004. Mitochondria in human oogenesis and preimplantation embryogenesis: engines of metabolism, ionic regulation and developmental competence. Reproduction 128, 269–280.

Van Blerkom, J., Davis, P., 2006. High-polarized (Delta Psi m(HIGH)) mitochondria are spatially polarized in human oocytes and early embryos in stable subplasmalemmal domains: developmental significance and the concept of vanguard mitochondria. Reprod. Biomed. Online 13, 246–254.

Van Blerkom, J., Davis, P., Alexander, S., 2000. Differential mitochondrial distribution in human pronuclear embryos leads to disproportionate inheritance between blastomeres: relationship to microtubular organization. ATP content and competence. Hum. Reprod. 15, 2621–2633.

Van Blerkom, J., Davis, P., Mathwig, V., Alexander, S., 2002. Domains of high-polarized and low-polarized mitochondria may occur in mouse and human oocytes and early embryos. Hum. Reprod. 17, 393–406.

Veitia, R.A., Nijhout, H.F., 2006. The robustness of the transcriptional response to alterations in morphogenetic gradients. BioEssays 28, 282–289.

Volodina, N., Denegre, J.M., Mowry, K.L., 2003. Apparent mitochondrial asymmetry in Xenopus eggs. Dev. Dyn. 226, 654–662.

Wenk, J., Brenneisen, P., Wlaschek, M., Poswig, A., Briviba, K., Oberley, T.D., Scharffetter-Kochanek, K., 1999. Stable overexpression of manganese superoxide dismutase in mitochondria identifies hydrogen peroxide as a major oxidant in the AP-1-mediated induction of matrix-degrading metalloprotease-1. J. Biol. Chem. 274, 25869–25876.

Wikramanayake, A.H., Hong, M., Lee, P.N., Pang, K., Byrum, C.A., Bince, J.M., Xu, R., Martindale, M.Q., 2003. An ancient role for nuclear beta-catenin in the evolution of axial polarity and germ layer segregation. Nature 426, 446–450.

Wilding, M., Carotenuto, R., Infante, V., Dale, B., Marino, M., Di Matteo, L., Campanella, C., 2001a. Confocal microscopy analysis of the activity of mitochondria contained within the 'mitochondrial cloud' during oogenesis in Xenopus laevis. Zygote 9, 347–352.

Wilding, M., Dale, B., Marino, M., di Matteo, L., Alviggi, C., Pisaturo, M.L., Lombardi, L., De Placido, G., 2001b. Mitochondrial aggregation patterns and activity in human oocytes and preimplantation embryos. Hum. Reprod. 16, 909–917.

Wylie, C., 1999. Germ cells. Cell 96, 165–174.

Yaffe, M.P., 1999. The machinery of mitochondrial inheritance and behavior. Science 283, 1493–1497.

Yoshida, S., Marikawa, Y., Satoh, N., 1996. Posterior end mark, a novel maternal gene encoding a localized factor in the ascidian embryo. Development 122, 2005–2012.

Yost, H.J., Phillips, C.R., Boore, J.L., Bertman, J., Whalon, B., Danilchik, M.V., 1995. Relocation of mitochondria to the prospective dorsal marginal zone during Xenopus embryogenesis. Dev. Biol. 170, 83–90.

Zalokar, M., Sardet, C., 1984. Tracing of cell lineage in embryonic development of Phallusia mammillata (Ascidia) by vital staining of mitochondria. Dev. Biol. 102, 195–205.

Zernicka-Goetz, M., 2002. Patterning of the embryo: the first spatial decisions in the life of a mouse. Development 129, 815–829.

Zernicka-Goetz, M., 2006. The first cell-fate decisions in the mouse embryo: destiny is a matter of both chance and choice. Curr. Opin. Genet. Dev. 16, 406–4012.

Zheng, L., Roeder, R.G., Luo, Y., 2003. S phase activation of the histone H2B promoter by OCA-S, a coactivator complex that contains GAPDH as a key component. Cell 114, 255–266.

Zima, A.V., Blatter, L.A., 2006. Redox regulation of cardiac calcium channels and transporters. Cardiovasc. Res. 71, 310–321.