HHS Public Access

Author manuscript

Genesis. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Genesis. 2017 January ; 55(1-2): . doi:10.1002/dvg.23007.

Maternal Messages to Live by: A personal historical perspective

Mary Lou King[1,*]

[1]Department of Cell Biology, University of Miami Miller School of Medicine, 1011 NW 15th St, Miami, FL 33136, USA

SUMMARY

In the 1980's, the study of localized maternal mRNAs was just emerging as a new research area. Classic embryological studies had linked the inheritance of cytoplasmic domains with specific cell lineages, but the underlying molecular nature of these putative determinants remained a mystery. The model system *Xenopus* would play a pivotal role in the progress of this new field. In fact, the first localized maternal mRNA to be identified and cloned from any organism was *Xenopus vg1*, a TGF-beta family member. This seminal finding opened the door to many subsequent studies focused on how RNAs are localized and what functions they had in development. *Xenopus* remains the model of choice for studies on dissecting RNA/protein transport particles and RNA-sequencing analyses that are moving the field into the future.

Keywords

Xenopus; localized RNAs; cytoplasmic determinants; germ line

Conceptual Background: Cytoplasmic domains, cell lineages and nuclear transplantation

There is a long and rich history of early embryologists documenting the relationship between cell fate and the inheritance of particular regions of the egg cytoplasm (Wilson, 1925). One of the most striking examples is that of the ascidian *Styela* that Conklin described in 1905. The *Styela* egg displayed a yellow crescent shaped region rich in mitochondria that was asymmetrically inherited by blastomeres that gave rise to the muscle cell lineage (Conklin 1905). Perhaps the first example in a vertebrate system linking a cytoplasmic domain to a specific lineage was the description by Blackler (1958) of germ plasm in the frog *Rana pipiens*. UV ablation of germ plasm led to the loss of the germline, a condition that could only be rescued by injecting cytoplasm containing germ plasm back into the vegetal pole (Smith, 1966). These experiments linked a visible structure, germ plasm, to an essential cell lineage and function (Williams and Smith, 1971). The biochemical nature of cytoplasmic determinants and their mode of action remained a mystery, although histological staining had suggested the presence of RNA (Bounoure, 1934).

*Author for correspondence (mking@med.miami.edu).

Coincidentally, developmental biologists were asking whether the process of differentiation was accompanied by the loss of genetic information. Put another way, were the nuclei of differentiated cells intrinsically equivalent to the egg genome and could differentiation be reversed. To address this question, Briggs and King (1952; 1960) had carried out a series of nuclear transplantation experiments injecting nuclei from advanced embryonic stages back into enucleated eggs of *Rana pipiens*. Their results had mixed success in promoting normal development, most likely because the chromosomes did not have sufficient time within the ooplasm to prepare for the rapid divisions imposed during cleavage stages after fertilization.

At this time, an important event occurred that would ultimately change the direction of this inquiry. Dr. Michail Fischberg asked his new student, John Gurdon, to repeat the Briggs and King nuclear transplantation experiments, but to use a different amphibian, *Xenopus laevis*. In 1966, John Gurdon showed beyond any reasonable doubt that indeed, nuclei from differentiated cells could completely support normal development when introduced into the enucleated egg (Gurdon and Uehlinger, 1966). These elegant experiments earned Sir John Gurdon the Nobel Prize in 2012 and showed that nuclei could be reprogrammed to a totipotent state. Similar to the cytoplasmic localization studies, they underscored the dominance of cytoplasmic information over nuclear expression. Sir Gurdon had also published papers showing that RNAs encoding proteins restricted to differentiated cells could be translated after injection into *Xenopus* oocytes (Gurdon et al., 1974; Woodland et al., 1974)! These experiments catapulted *Xenopus* onto the world stage as a model embryological system for studying gene expression during development. I was a student in Robert Briggs's lab in the 1970's working with *Rana pipiens* as well as axolotls to address this question of developmental plasticity. I remember well meeting Sir John Gurdon during his visit to Brigg's lab where they discussed the different outcomes of their nuclear transplantation results. In hindsight, I realized I learned valuable lessons from this part of my professional history: It is critical to choose the right model system to answer the question being asked and important to remain working in your lab as a PI. I have always admired John Gurdon for doing that and setting an example.

The Xenopus Oocyte: Identifying localized maternal RNAs

In 1976, I headed to MIT and Harvey Lodish's lab to learn molecular cloning, a new technology at the time. After working with *Rana pipiens* and *axolotls*, I was looking for a simpler model system in which to understand gene regulation during development. *Dictyostelium* seemed a good choice at the time as there were only two cell types: stalk versus spore. Since these two presumptive cell types were physically separated from each other in the migrating slug, my thought was to cut thousands of these slime mold slugs, isolate RNA from the different regions and screen for differences. Wrestling with exactly how to do this, I realized how much easier the task would be using the large and visually polarized *Xenopus* oocyte instead.

In truth, I missed the beauty and embryological history of frog development: "How the embryonic body axis emerges from a seemingly symmetrical egg and is subsequently patterned during embryogenesis is one of the most fundamental questions in developmental biology" (Scott Gilbert). I was not going to answer that question working on *Dictyostelium*.

After one year, I asked Harvey if I could bring *Xenopus* into the lab as a model system and work on translational control with a focus on maternal RNAs. He had a well-established reputation in the field of translation, and he agreed. I went on to show that different RNAs were translated during the progression of *Xenopus* oogenesis while some remained translationally silent. During this time, I learned *Xenopus* husbandry, the use of in vitro translation systems and protein analyses by 2-dimentional gel electrophoresis, all of which would serve me well in my own lab. Most importantly, I spent an intense time in the library going through the literature on the maternal contribution to early development and prepared to write my first grant to NSF. Little did I know that across the Charles River at Harvard a former graduate student of John Gurdon, Doug Melton, was thinking the same thoughts as I was: there must be vegetally localized maternal mRNAs that drove early patterning of the *Xenopus* embryo.

Looking back, the time I spent in the library reading and thinking about what problems I wanted to tackle was worth every minute! What I decided to write my grant about during this time would end up being a new field that would consume the rest of my professional career.

The rationale driving the search for localized maternal mRNAs was straightforward. Zygotic transcription did not begin until the embryo was at the mid-blastula stage and 4,000 cells (Newport and Kirschner, 1982). Yet three basic developmental decisions had been made that were known to initiate at the vegetal pole: the dorsal/ventral (reviewed in Weaver and Kimelman, 2004; Houston, 2012) and primary germ layer identities (Nieuwkoop, 1977) as well as the germ cell determinants in the form of germ plasm (Smith, 1966). Therefore, maternal transcripts must be involved and regionally localized within the egg. Historically, a major obstacle to examining the spatial distribution of individual mRNAs had been the inability to prepare specific cytoplasmic regions of eggs in quantities suitable for biochemical analysis. The fully grown *Xenopus* oocyte is visibly polarized along the important animal/vegetal axis with cortical melanosomes at the animal hemisphere and relatively few vegetally. A single oocyte at 1.3 mm contains ~4ug of total RNA. The first set of experiments I did after starting my own lab exploited the clear advantages of *Xenopus*. Within cryostat embedding medium, we aligned thousands of oocytes along their A/V axis and cut frozen sections essentially dividing a single cell into three regions. RNA was isolated from these frozen sections.

Previous to our experiments, Joel Richter and colleagues had shown that as much as 70% of the oocyte's cytoplasmic poly(A)+RNA was untranslatable. "The experiments we have presented provide no support for the proposal that the interspersed poly(A) RNAs are true maternal messages, meaning molecules capable of being directly loaded on polysomes and translated during maturation or development." (from Richter et al., 1984) From this statement, we worried about selecting non-translatable RNAs. To avoid this potential problem, we decided to translate the mRNA isolated from sectioned oocytes using in vitro translation systems, and analyze the protein products on 2-D gels looking for differences along the A/V axis. We found these differences and published the results in Developmental Biology (King and Barklis, 1985). The big disadvantage of our approach was that we did not know the identity of the proteins differentially translated.

Meanwhile, Doug Melton was cutting tips off oocytes with a scalpel that were frozen in their jelly coats. In 1984 he had published a transformative methods paper on how to transcribe mRNAs in vitro (Krieg and Melton, 1984). Doug screened a cDNA oocyte library made from mRNA transcripts for clones highly enriched at either the animal or vegetal pole. Four localized mRNAs were discovered, three at the animal pole and one at the vegetal pole (Rebagliati et al., 1985). He named the vegetal pole mRNA *vg1 (gdf1)*. Importantly, they showed that *vg1* (gdf1) mRNA remained localized into cleavage stages and thus could potentially influence cell fate. Having cloned *vg1(gdf1)* they were now in position to sequence it and determine its identity (Weeks and Melton, 1987). Excitedly, Vg1 (Gdf1) proved to be a TGF-beta member and, as a known signaling molecule, it's likely function in patterning the early embryo seemed obvious. However, it would take another eighteen years before the endogenous function would be assigned to Vg1 (Gdf1) (Birsoy et al., 2005). Doug had anticipated the need to visualize the localization process and developed in situ hybridization in *Xenopus*. Using this new technique, *vg1 (gdf1)* was shown to be tightly associated with the oocyte vegetal cortex and released after fertilization where it was inherited by blastomeres comprising the endoderm (Weeks and Melton, 1987). Doug was able to published two papers in Cell and I published one in Developmental Biology. In hindsight, I should have just gone straight to cloning and not worried about translation! The image of localized *vg1 (gdf1)* made the cover of Cell in 1987 and laid the foundation for answering the next set of questions: what other mRNAs were localized, how were they localized, and what functions did they have?

Germ Plasm RNAs: The search for more localized maternal mRNAs

The tight cortical localization of *vg1 (gdf1)* mRNA pointed to the cytoskeleton as the likely agent for retaining RNAs at one pole. To test this idea, my lab extracted oocytes in a high salt detergent solution that left basically only cytokeratins in an insoluble pellet. We were surprised to find that the pellet contained virtually all the oocyte *vg1 (gdf1)* mRNA (Pondel and King, 1988). In the Weeks and Melton paper they had shown *vg1 (gdf1)* mRNA was no longer retained within the cortex, but released to occupy the vegetal blastomeres. Consistent with that observation, we found that after fertilization, *vg1 (gdf1)* mRNA was no longer recovered from the cytoskeletal fraction but was now found in the soluble fraction. This result was foreshadowed by Jeffery and Meier (1983) who showed that actin mRNA was retained in a detergent extracted *Styela* embryo at the posterior pole of the 2-cell embryo. Our discovery was important for two reasons: it focused attention on the intermediate filaments as part of the anchoring complex for mRNA; and 2) it pointed the way to biochemically isolating additional localized maternal mRNAs. We reasoned that if the oocyte cytoskeletal matrix serves to anchor mRNAs, then other localized maternal mRNAs should also be highly enriched in the intermediate filament fraction (IFF).

We generated a lambda cDNA library from the IFF sample and probed this library with mRNA highly enriched in the IFF. Clones that hybridized only to the IFF mRNA but not soluble mRNAs were isolated. This strategy was successful and we named the clones *Xenopus* cytoskeletal associated transcripts or *xcats*. Our first clone, *xcat2*, was identified as being the *Xenopus* homolog to the *Drosophila nanos*. As *nanos* was essential in the germline, these results pointed to conserved functions between invertebrate and vertebrate

germlines. I presented these findings in a poster at the Developmental Biology meetings and I remember Ruth Lehmann being very happy that we had established this link. *xcat3* was also found in the germ plasm and identified as one of the germline helicases. We named it *deadsouth (ddx25)* (Mosquera et al., 1993; MacArthur et al., 2000). The *deadsouth (ddx25)* 3'UTR is currently used to target transcripts to the germ line.

Rick Elinson had been working on the relationship between microtubule arrays, cortical rotation, and dorsal axis specification in *Xenopus* (Elinson and Rowning, 1988). He had developed a method for isolating structurally intact cortices from either the animal or vegetal pole of oocytes. Ultrastructural and immunocytochemical analyses revealed that the 10-μm-thick isolates included a cytokeratin network, mitochondria, and other elements, found in the oocyte cortex. He suggested we determine if *vg1(gdf1)*, *xcat2 (nanos1)* and *xcat3 (ddx25)* co-localize with the vegetal cortices. All three vegetally localized mRNAs were found to be specifically retained within the isolated vegetal cortices, in sharp contrast to histone RNA. These findings supported the view that the vegetal cortex represented a unique cytoskeletal domain in which a class of maternal mRNAs became localized (Elinson et al., 1993). In that same year, Larry Etkin's group published a paper in Science describing *xlsirts*, or interspersed repetitive transcripts that localized to the mitochondrial cloud. Janet Heasman and Chris Wylie had shown in 1984 that the germ plasm formed within the mitochondrial cloud (MC) also called Balbiani body. *xlsirts* were the first RNAs to be clearly shown by in situ hybridization to be part of the germ plasm (Kloc et al., 1993). *xlsirts* represented hundreds of RNAs, believed to be non-coding RNAs related to the X-chromosome inactivation *xist* gene. The role of non-coding RNAs within the germ plasm generated a lot of speculation.

xwnt11 was also found to localize within the MC (Ku and Melton, 1993), a surprise as *xwnt11* was thought to play a role in axial patterning based on its ability to induce a secondary axis (McMahon and Moon, 1989; Smith and Harland, 1991; Sokol et al., 1991). Meanwhile, John Gerhart, Randy Moon and Carolyn Larabell were investigating the molecular basis for cortical rotation and dorsal specification, that occurs before first cleavage. They showed that vesicles were moved via microtubules during cortical rotation, an event required for the localization of β-catenin protein to the future dorsal side of the *Xenopus* egg (Rowning et al., 1993) and critical to dorsal specification (Heasman et al., 1994). The role of *xwnt11* in dorsal specification would not be confirmed until 2005 (Tao et al., 2005). The role of x*wnt11* in the germline, if any, has never been determined.

We were excited to determined the spatial pattern of expression for *nanos1 (xcat2)* in *Xenopus* because it had been shown to be exclusive to the *Drosophila* germline. Would the localization pattern be different from that of *vg1(gdf1)* mRNA? Indeed, it was, localizing very early during oogenesis to the MC, and well before *vg1 (gdf1)* mRNA would begin its microtubule based transport to the vegetal cortex. Our work defined two localization pathways: an Early germline pathway followed by *nanos1* and *xlsirts*, and a Late pathway defined by *vg1 (gdf1)* (Forristall et al., 1995). A month later, Kloc and Etkin published similar findings, but with a much better title, clearly stating the result (Kloc and Etkin, 1995). The valuable lesson I learned was that titles matter as they do impact the number of times your work is referenced. I also learned that no one owns a field just because you were

the first one on the scene. Etkin's lab came to refer to the Early MC pathway as METRO for Message Transcript Organizer.

We and the Etkin lab mapped the 3'UTR sequences required for mRNAs to localize within the MC and discovered that the localization signal was made up of two elements. One element was required for *nanos1* mRNA to concentrate within the MC while the second element was required for its germinal granule localization (Zhou and King, 1996a,b; Kloc et al., 2000). Over the next few years, investigators in the field focused on both identifying more localized mRNAs and the mechanism of mRNA localization. Hugh Woodland screened a gastrula vegetal pole cDNA library and discovered *xpat (pgat)*, one of the few mRNAs to be exclusively expressed in PGCs. *xpat (pgat)* has been an invaluable marker for primordial germ cells (PGCs) (Hudson and Woodland, 1998). Xpat (Pgat) is also a major protein component of the germ plasm and provided the first glimpse into the nature of the germ plasm matrix (Machado et al., 2005). Hugh Woodland's lab went on to discover more mRNAs expressed in PGCs using this screening approach (reviewed in King, 2014; Houston, 2013).

The Etkin lab isolated mitochondrial clouds using gradient methods. Libraries made from MC mRNA yielded additional germ plasm mRNAs including *fatvg (plin2)* and *hermes (rbpms)* (Chan et al., 1999; Zearfoss et al., 2004). Interestingly, *fatvg (plin2)* and *hermes (rbpms)* used both the early (METRO) and Late pathways suggesting overlapping or redundant elements. During this time, several labs realized that mRNAs normally localized to the MC, could also use the Late pathway if injected at later stages. The same localization signals appeared to function early or late. Confusion ruled as to just what a localization signal was and how different pathways were chosen endogenously.

Mechanism of RNA localization

When Doug Melton accepted his academic position at Harvard, he insisted on having his own lab space within his office, all set up to do experiments. He wanted to protect his time at the lab bench. The fruit of that decision was clear in his next work published in Nature as sole author (Melton 1987). Here he showed for the first time that mRNA localization occurred progressively during oogenesis using a mechanism that did not require prior translation or mRNA degradation/protection. *vg1 (gdf1)* RNA injected into cultured oocytes recapitulated normal localization to the vegetal cortex, opening up an avenue for studying the mechanism involved (Yisraeli and Melton, 1988). It was quickly established that *vg1 (gdf1)* localization required intact microtubules and actin filaments for cortical retention (Yisraeli et al., 1990).

Could an mRNA localization signal be defined? Kim Mowry joined the Melton lab and identified by deletion mapping the required sequences for localization to occur. A 340-nt piece in the 3'UTR was required and sufficient for normal localization (Mowry and Melton, 1992). She was now in a position to find the proteins that interacted with this sequence and define the localization machinery. Further refining of the localization signal into elements recognized a repeated cluster of the pentanucleotide UUCAC that seemed to be important (Deshler et al., 1997; Bubunenko et al., 2002). The Mowry lab went on to define

microtubule motors involved and associated mRNA binding proteins that comprised the *vg1 (gdf1)* transport particle. Key findings during this time were 1) that mRNA binding proteins normally thought to be strictly nuclear (like hnRNPI) were part of mRNA transport particles, remaining with mRNAs as they were localized (Cote et al., 1999); and 2) the steps that occur as mRNAs are identified in the nucleus for transport and the remodeling that occurs as mRNAs enter the cytoplasm and associate with microtubules (Lewis et al., 2008; Kress et al., 2004), 3) the microtubule motors involved in mRNA transport (Gagnon et al., 2013; Messitt et al., 2008). The Thomas Pieler lab, using an affinity purification approach, also contributed to identifying RNP components and localization signals required for vegetal localization. Importantly, his lab identified Elr-type proteins that associate with mRNA localization elements, helping to stabilize mRNAs within the germline from somatic clearance by *miR-18*. Their work provided a functional link of vegetal mRNA localization and the protection (Koebernick et al., 2010).

In 1996, Larabell and Etkin's labs moved towards a mechanistic understanding of mRNA localization into the MC taking full advantage of the complete transparency of stage I oocytes. They synthesized fluoroscein- or Texas red- labeled RNAs (*xlsirt*, *xcat2 (nanos)*, and *xwnt11*) using derivatized nucleotides and injected these synthetic mRNAs into stage I transparent oocytes. They found that the translocation of RNAs through the early or METRO pathway, unlike that of the late pathway, occurred in the absence of intact microtubule and actin microfilament cytoskeletal elements. Inspired by this work, we collaborated with Evelyn Houliston to make movies of *nanos1* (*xcat2*) MC mRNA localization. We showed this event occurred by diffusion through the ooplasm and entrapment on the very dense endoplasmic reticulum that occupied the MC (Chang et al., 2004). The connection between mRNA localization and the endoplasmic reticulum has never been resolved (Kloc et al., 1998).

Function of localized maternal messages

The ability to actually peel the cortex from *Xenopus* oocytes offered a unique advantage for isolating and identifying additional localized mRNAs. Probing the IFF cDNA library with mRNAs isolated from either animal or vegetal hand-isolated cortices, Jian Zhang in my lab identified five novel vegetal pole mRNAs. Excitingly, the screen identified the first localized transcription factor, *vegt*, in the T-box family. Its expression pattern was entirely consistent with a regulatory role in endoderm specification. We were anxious to move forward and determined its function as no less than three other labs had also isolated the same gene, all naming it differently: the Gurdon lab called it *antipodean* (Stennard et al., 1996); the Thomsen lab called it *brat* (Horb and Thomsen, 1997) and the Kirschner lab called it *xombi* (Lustig et al., 1996).

At that time, we had poor tools for determining the function of these localized maternal mRNAs. The most useful approach took advantage of the RNAse H activity in oocytes that degrades RNA-DNA hybrids. mRNAs could be targeted for degradation after injection of specific antisense cDNA oligodeoxynucleotides, or ODN (oligos). The other main antisense reagent for loss-of-function experiments came in 1997 with the advent of morpholinos oligos. These do not degrade the message but have the potential to form stable hybrids that

block translation or splicing of the transcript (Summerton et al., 1997). Janet Heasman and Chris Wylie had developed a procedure commonly referred to as the Host Transfer Technique, that would allow a direct interrogation of function of maternal components. They had successfully depleted maternal cytokeratin mRNA from oocytes and generated a loss-of-function phenotype in resulting embryos (Torpey et al., 1992). Host transfer remains the only way to deplete maternal information to determine function. Fortuitously, Janet and I were both at the 1996 *Xenopus* International Conference at Estes Park, Colorado. I approached her to collaborate on a *vegt* project and she enthusiastically agreed. As it turned out, John Gurdon also asked her to collaborate shortly thereafter. The findings that resulted from our collaboration were quite stunning and this time period was one of the most exciting in my career. Our work defined *vegt* as the endoderm determinant and showed that it was required for mesoderm induction (Zhang et al., 1998). Other mRNAs that were discovered in Jian Zhang's screen besides *vegt* included: *xdazl*, a long chain acyl-CoA synthetase the depletion of which causes meiotic arrest; *arh* for autosomal recessive hypercholesterolemia; and *fingers,* a zinc finger repressor (Zhang and King, 1996; Houston et al., 1998; Houston and King, 2000; Zhou et al., 2003; Wang et al., 2012; Venkataraman et al., 2004). The lesson was clear: Do not hesitate to collaborate with those that can quickly move a project forward.

Janet Heasman went on to solve other mysteries surrounding localized maternal mRNAs using the Host Transfer methods she developed. I remember the long running attempts of Doug Melton and colleagues to show the endogenous function for the protein product of *vg1* RNA. They were only able to show what it was capable of doing when it's expression was forced. However, it turned out they had been using a *vg1 (gdf1)* allele carrying a mutation that failed to be efficiently cleaved which was required for its activation. Janet Heasman and colleagues isolated the active v*g1 (gdf1)* allele and went on to show that Vg1 was required for mesoderm induction and for the expression of several key BMP antagonists. The lessons learned from the *vg1 (gdf1)* saga are: to make sure you have the correct allele, and continual failure may be trying to tell you something.

Anchoring localized mRNAs to the cortex: The concept of binary functions for mRNAs

Larry Etkin had previously shown that disruption of non-coding *xlsirt* RNAs caused other localized mRNAs to lose their cortical localization (Kloc et al., 2005). In 2001 a former postdoctoral student of Doug Melton, Dan Kessler, published a paper that rocked our world (Heasman et al., 2001). He had depleted *vegt* mRNA and found that it caused the release of *vg1 (gdf1)* mRNA from the vegetal cortex. The total amount of *vg1 (gdf1)* mRNA was not affected; however, a reduction of Vg1 protein was observed. Other mRNAs were affected in a similar fashion (Bicaudal-C and Wnt11) while others were not (*nanos1 (Xcat2), xotx1*). Were our results defining the function of *vegt* incorrect, and depletion was really dislodging *vg1* mRNA and that explained the loss of endoderm/mesoderm fate? Fortunately, there was a way to tell. Dislodging *vg1 (gdf1)* depended on *vegt* mRNA and not protein as a morpholino that blocks translation while leaving the mRNA intact, had no effect. These results indicated a novel structural function for maternal *vegt* mRNA separate from its protein coding

function. Lessons learned: mRNAs may not just code for proteins! Be careful in your interpretations of anti-sense RNA destruction experiments.

Earlier observations had hinted at a role for cytokeratins in mRNA cortical anchoring, but little progress had been made since then (Pondel and King, 1988; Alacron and Elinson, 2001). But in 2005, the Etkin lab published an important paper showing that the organization of the cytokeratin filaments, but not the actin cytoskeleton, depended on *vegt* RNA and *xlsirts* (Kloc et al., 2005; 2007). Very sadly, in March of 2006, Larry Etkin died at the age of 61. His lab was dissolved, but Malgorzata Kloc found a new position and carried on with these studies at an ultrastructural level (Kloc et al., 2011a; reviewed in Kloc et al., 2011b).

Current search for maternal localized RNAs and their functions

Most recently, various labs have revisited the search for novel localized mRNAs, now bringing next generation sequencing to the task. RNA-seq analysis has the distinct advantage of cataloging all RNA sequences whether coding or non-coding. It provides a data base that can be mined again and again as the annotation of the *Xenopus* genome improves. These efforts have identified hundreds of new mRNAs enriched along the animal/vegetal axis, with only about half annotated, leaving much work to be done (De Domenico et al., 2015; Claussen et al., 2015; Owens et al., 2017; reviewed in Houston 2013). Interestingly, no biases in mRNAs could be detected along the dorsal/ventral or right/left axis at the 8-cell stage (De Domenico et al., 2015). General themes that are emerging from this line of investigation emphasize the vegetal pole as a major hub for signaling and directing early patterning in the embryo. Comparisons between the two poles show much less complexity and enrichment at the animal pole. Interestingly, many vegetally localized mRNA were also expressed in neural ectoderm derivatives (Owens et al., 2017). Our efforts have focused on the germ cell lineage and developing a catalog of all mRNAs and proteins found in the germ plasm beginning with mitochondrial clouds, vegetal cortical germ plasm, and germ plasm in PGCs after they have segregated from the endodermal lineage. *Xenopus* offers unique advantages in developing such an inventory. Mitochondrial clouds from stage I oocytes are very large structures, 30 microns in diameter, and can be hand-isolated in large enough numbers that make mass spectrometry and RNA-seq analyes practical. Vegetal cortices from fully grown oocytes and pure populations of PGCs also are possible to isolate in quantities for biochemical analyses. Soon we will have a comprehensive list of RNAs and proteins operating within the germ plasm from formation to lineage separation during early development. We look forward to more surprises as to how the germ cell lineage maintains full potential within the developing embryo.

FUTURE DIRECTIONS

Of course, there are many questions that remain to be answered regarding the regulatory networks operating at the vegetal pole as well as the mechanisms of mRNA localization. Now that more sophisticated methods and techniques are available for *Xenopus* and are being continuously developed, the rate of progress will undoubtedly accelerate. Functional studies can now take advantage of CRISPR/Cas9 (Blitz et al., 2013; Nakayama et a., 2013), transgenic lines expressing tagged maternal mRNAs, improved sequencing and annotation,

time released and spatially targeted inhibitors (detailed in Hoppler and Vize (eds.), 2012). To aid the search for RNA binding proteins-RNA complexes there is CLIP technology combined with high throughput sequencing (Ray et al., 2013). We now have significantly better structure prediction tools for RNA (Ahmad et al., 2013) and the ability to determine protein binding within the context of relevant RNA 3-D structures (Schroder et al., 2010).

Here is my personal list of areas that I believe would be exciting to pursue.

Vegetal Pole as an Organizing Center for establishing cell fates

a. Evidence from RNA-seq analyses suggest that the canonical Wnt pathway is not the only Wnt pathway operating at the vegetal pole. Components for the PCP pathway are also present suggesting a more integrated, convergent Wnt pathway functioning at the vegetal pole (Owens et al., 2017). *xwnt11* mRNA is found in the germ plasm and may play as yet an undescribed role during PGC and/or neural crest migration. Perhaps the integration of these Wnt pathways would explain why several genes when knocked down, affect both dorsal specification and PGC development (Colozza and De Robertis, 2014; Chan et al., 1999).

b. The identity of the dorsal determinant remains unknown.

c. Microtubule array formation critical for cortical rotation and dorsal specification is regulated specifically within the vegetal hemisphere. What are the molecular components that regulate microtubule dynamics spatially?

d. What are the maternal gene networks inherited by the PGCs that allow full potential to be retained within the context of other signaling pathways? We have begun this analysis by comparing RNA-seq and mass spectrometry data from isolated PGCs to determine the maternal and zygotic contributions to this lineage. How are PGC mRNAs controlled at the translation level?

e. A recurrent theme is how common vegetally localized mRNAs also play a role in neurogenic fates or functions (Owens et al., 2017). In fact, the most abundant mRNA within the vegetal cortex is *grip2* (glutamate receptor interacting protein), a scaffold multiple PDZ domain protein that organizes signaling complexes in neurons (Kaneshiro et al., 2007; Ge et al., 2014; Claussen et al., 2011; Tarbashevich et al., 2007; Tan et al., 2015). Grip2 protein is present within vegetal cortices and may well serve as a scaffold for signaling at the vegetal pole providing the link between Wnt and PGC migration (unpublished observations, Newman and King). We suggest that the nervous system uses many of the same components when a signaling scaffold and cell migration are required.

f. Are there LncRNAs at the vegetal pole and if so, what roles do they play? *xlsirts* were discovered back in the early 90's, but further studies have waited for better methods of sequence and annotation. Now that the technology has improved significantly, real progress can be made answering these questions about non-coding RNAs.

g. Are there DNA modifying enzymes that could spatially regulate epigenetic changes in the early embryo?

Mechanisms for RNA Localization

mRNA localization is a fundamental cellular process with profound effects for function in a wide variety of cells including the oocyte. The *Xenopus* oocyte has provided a powerful model system to study both the early and late pathways, but major gaps still exist in our understanding.

a. What defines an mRNA localization signal? We still do not know the underlying principles that regulate RNA-protein interactions. Deletion/functional mapping of mRNA localization signals has not really defined a consensus sequence for localization although certain motifs seem to be important, but not all the time. Secondary RNA structure must be playing an important role.

b. What are the nuclear events that initiate mRNA localization? What determines localization specificity?

c. How to localization signals recruit microtubule motors? Do different mRNAs form their own particles for transport? Are non-coding RNAs involved?

d. What is the role of the ER and other organelles (mitochondria, lipid droplets) in mRNA localization? Do they provide a scaffold for mRNAs, but how?

e. How does RNA act as a structural element with the cytoskeleton?

The field can anticipate surprises ahead as these questions and more are tackled.

Acknowledgments

One can never forget all those who came before that laid the conceptual foundation and leadership in your field. My heartfelt gratitude goes out to them. To those who over the years have become friends as well as colleagues, I am grateful for the sharing of ideas, reagents, and good times at meetings. I would also like to acknowledge all the members of my lab, past and present, for making my research experience so rewarding. Special thanks go to Drs. Tristan Aguero and Karen Newman for help in completing this article. This work was supported by NIH HD072340 and GM102397 (MLK).

LITERATURE CITED

Ahmad F, Mahboob S, Gulzar T, Din SU, Ahmad H, Afzal M. RNA-SSPT: RNA secondary structure prediction tools. Bioinformatics. 2013; 9:873–878.

Alarcon VB, Elinson RP. RNA anchoring in the vegetal cortex of the Xenopus oocyte. J Cell Sci. 2001; 114:1731–1741. [PubMed: 11309203]

Birsoy B, Berg L, Williams PH, Smith JC, Wylie CC, Christian JL, Heasman J. XPACE4 is a localized pro-protein convertase required for mesoderm induction and the cleavage of specific TGFbeta proteins in *Xenopus* development. Development. 2005; 132:591–602. [PubMed: 15634697]

Blackler AW. Contribution to the study of germ-cells in the anura. J Embryol Exp Morphol. 1958; 6:491–503. [PubMed: 13575662]

Blitz IL, Biesinger J, Xie X, Cho K. Biallelic genome modification in F0 *Xenopus tropicalis* embryos using the CRISPR/Cas system. Genesis. 2013; 51:827–834. [PubMed: 24123579]

Bounoure L. Recherches sur la lignée germinale chez la grenouille rousse aux premiers stades du développment. Ann Sci Nat. 1934; 17:67–248.

Briggs R, King TJ. Tranplantation of living nuclei from blastula cells into enucleated frogs' eggs. Proc Natl Acad Sci USA. 1952; 38:455–463. [PubMed: 16589125]

Briggs R, King TJ. Nuclear transplantation studies on the early gastrula (*Rana pipiens*). I. Nuclei of presumptive endoderm. Dev Biol. 1960; 2:252–270. [PubMed: 13804423]

Bruce AE, Howley C, Zhou Y, Vickers SL, Silver LM, King ML, Ho RK. Organizer formation and early cell movements are regulated by the maternally expressed zebrafish T-box gene, eomesodermin. Development. 2003; 130:5503–5517. [PubMed: 14530296]

Bubunenko M, Kress TL, Vempati UD, Mowry K, King ML. A consensus RNA signal that directs germ layer determinants to the vegetal cortex of *Xenopus* oocytes. Dev Biol. 2002; 248:82–92. [PubMed: 12142022]

Chan AP, Kloc M, Etkin LD. *fatvg* encodes a new localized RNA that uses a 25-nucleotide element (FVLE1) to localize to the vegetal cortex of *Xenopus* oocytes. Development. 1999; 126(22):4943–4953. [PubMed: 10529413]

Chang P, Torres J, Lewis R, Mowry K, Houlison E, King ML. Localization of RNAs to the mitochondrial cloud in Xenopus oocytes by entrapment and association with endoplasmic reticulum. Mol Biol Cell. 2004; 15:4669–4681. [PubMed: 15292452]

Claussen M, Lingner T, Pommerenke C, Opitz L, Salinas G, Pieler T. Global analysis of asymmetric RNA enrichment in oocytes reveals low conservation between closely related *Xenopus* species. Molecular Biology of the Cell. 2015; 26:3777–3787. [PubMed: 26337391]

Claussen M, Tarbashevich K, Pieler T. Functional dissection of the RNA signal sequence responsible for vegetal localization of *xgrip2*.1 mRNA in *Xenopus* oocytes. RNA Biol. 2011; 8(5):873–882. [PubMed: 21788733]

Colozza G, De Robertis EM. Maternal syntabulin is required for dorsal axis formation and is a germ plasm component in *Xenopus*. Differentiation. 2014; 88:17–26. [PubMed: 24798204]

Conklin EG. Mosaic development in *ascidian* eggs. J Exp Zool. 1905; 2:145–223.

Cote CA, Gautreau D, Denegre JM, Kress TK, Terry NA, Mowry KL. A *Xenopus* protein related to hnRNPI has a role in cytoplasmic RNA localization. Mol Cell. 1999; 4(3):431–437. [PubMed: 10518224]

Cui Y, Brown JD, Moon RT, Christian JL. Xwnt8b: A maternally expressed *Xenopus wnt* gene with a potential role in establishing the dorso-ventral axis. Development. 1995; 121:2177–2186. [PubMed: 7635061]

Cuykendall TN, Houston DW. Identification of germ plasm-associated transcripts by microarray analysis of *Xenopus* vegetal cortex RNA. Dev Dyn. 2010; 239:1838–1848. [PubMed: 20503379]

DeDomenico E, Owens ND, Grant IM, Gomes-Faria R, Gilchrist MJ. Molecular asymmetry in the 8-cell stage *Xenopus tropicalis* embryo described by single blastomere transcript sequencing. Dev Biol. 2015; 408:252–268. [PubMed: 26100918]

Deshler JO, Highett MI, Schnapp BJ. Localization of *Xenopus vg1* mRNA by Vera protein and the endoplasmic reticulum. Science. 1997; 276:1128–1131. [PubMed: 9148809]

Elinson R, King ML, Forristall C. Isolated vegetal cortex from *Xenopus* oocytes selectively retains localized mRNAs. Dev Biol. 1993; 160:554–562. [PubMed: 8253283]

Elinson RP, Rowning B. A transient array of parallel microtubules in frog eggs: potential tracks for a cytoplasmic rotation that specifies the dorso-ventral axis. Dev Biol. 1988; 128:185–197. [PubMed: 3289985]

Forristall C, Pondel M, Chen L, King ML. Patterns of localization and cytoskeletal association of two vegetally localized RNAs, *vg1* and *xcat-2*. Development. 1995; 121:201–208. [PubMed: 7867501]

Gagnon JA, Kreiling JA, Powrie EA, Wood TR, Mowry KL. Directional transport is mediated by a Dynein-dependent step in RNA localization pathway. PLoS Biol. 2013; 11(4):e1001551. [PubMed: 23637574]

Ge X, Grotjahn D, Welch E, Lyman-Gingerich J, Holguin C, Dimitrova E, Abrams EW, Gupta T, Marlow FL, Yabe T, Adler A, Mullins MC, Pelegri F. Hecate/Grip2a acts to reorganize the cytoskeleton in the symmetry-breaking event of embryonic axis induction. PLoS Genet. 2014; 10(6):e1004422. Erratum in: PLoS Genet 10(10):e1004786. [PubMed: 24967891]

Grant P, Wacaster JF. The amphibian gray crescent region--a site of developmental information? Dev Biol. 1972; 28(3):454–471. [PubMed: 4537788]

Gurdon JB, Colman A. The future of cloning. Nature. 1999; 402:743–746. [PubMed: 10617195]

Gurdon JB, Uehlinger V. “Fertile” intestine nuclei. Nature. 1966; 210:1240–1241. [PubMed: 5967799]

Gurdon JB, Woodland HR, Lingrel JB. The translation of mammalian globin mRNA injected into fertilized eggs of *Xenopus laevis*. I. Message stability in development. Dev Biol. 1974; 39:125–133. [PubMed: 4471721]

Heasman J, Crawford A, Goldstone K, Garner-Hamrick P, Gumbiner B, McCrea P, Kintner C, Noro CY, Wylie C. Overexpression of cadherins and underexpression of β-catenin inhibit dorsal mesoderm induction in early *Xenopus* embryos. Cell. 1994; 79:791–803. [PubMed: 7528101]

Heasman J, Wessely O, Langland R, Craig EJ, Kessler DS. Vegetal localization of maternal mRNAs is disrupted by *vegT* depletion. Dev Biol. 2001; 240:377–386. [PubMed: 11784070]

Hoppler, S., Vize, P., editors. Methods in Molecular Biology. *Xenopus* Protocols: Post-genomic approaches. Second. New York, NY: Springer; 2012.

Horb M, Thomsen G. A vegetally localized T-box transcription factor in *Xenopus* eggs specifies mesoderm and endoderm and is essential for embryonic mesoderm formation. Development. 1997; 124:1689–1698. [PubMed: 9165117]

Houston DW. Cortical rotation and messenger RNA localization in *Xenopus* axis formation. Dev Biol. 2012; 1(3):371–388.

Houston DW. Regulation of cell polarity and RNA localization in vertebrate oocytes. International review of cell and molecular biology. 2013; 306:127–185. [PubMed: 24016525]

Houston DW, King ML. A critical role for *xdazl*, a germ plasm-localized RNA, in the differentiation of primordial germ cells in *Xenopus*. Development. 2000; 127:447–456. [PubMed: 10631166]

Houston D, Zhang J, Maines J, Wasserman S, King ML. A *Xenopus* DAZ-like gene encodes an RNA component of germ plasm and is a functional homologue of *Drosophila boule*. Development. 1998; 125:171–180. [PubMed: 9486791]

Hudson C, Woodland H. *Xpat*, a gene expressed specifically in germ plasm and primordial germ cells of *Xenopus laevis*. Mech Dev. 1998; 73:159–168. [PubMed: 9622619]

Jeffery WR, Meier S. A yellow crescent cytoskeletal domain in ascidian eggs and its role in early development. Dev Biol. 1983; 96:125–143. [PubMed: 6186551]

Kaneshiro K, Miyauchi M, Tanigawa Y, Ikenishi K, Komiya T. The mRNA coding for *Xenopus* glutamate receptor interacting protein 2 (XGRIP2) is maternally transcribed, transported through the late pathway and localized to the germ plasm. Biochem Biophys Res Commun. 2007; 355(4): 902–906. Erratum in: Biochem Biophys Res Commun 365(4):891. [PubMed: 17320814]

King, ML. Germ cell Specification in *Xenopus*. In: Kloc, M., Kubiak, JZ., editors. *Xenopus* Development. Hoboken, New Jersey: John Wiley & Sons, Inc; 2014. p. 75-100.

King ML, Barklis E. Regional distribution of maternal messenger RNA in the amphibian oocyte. Dev Biol. 1985; 112:203–212.

Kirilenko P, Weierud FK, Zorn AM, Woodland HR. The efficiency of *Xenopus* primordial germ cell migration depends on the germplasm mRNA encoding the PDZ domain protein Grip2. Differentiation. 76(4):392–403.

Kloc M, Bilinski S, Dougherty MT. Organization of cytokeratin cytoskeleton and germ plasm in the vegetal cortex of *Xenopus laevis* oocytes depends on coding and non-coding RNAs: three-dimensional and ultrastructural analysis. Exp Cell Res. 2007; 313:1639–1651. [PubMed: 17376434]

Kloc M, Bilinski S, Pui-Yee Chan A, Etkin LD. The targeting of *xcat2* mRNA to the germinal granules depends on a cis-acting germinal granule localization element within the 3'UTR. Dev Biol. 2000; 217:221–229. [PubMed: 10625548]

Kloc M, Dallaire P, Reunov A, Major F. Structural messenger RNA contains cytokeratin polymerization and depolymerization signals. Cell Tissue Research. 2011a; 346:209–222. [PubMed: 21987223]

Kloc M, Dougherty MT, Bilinski S, Chan AP, Brey E, King ML, Patrick CW Jr, Etkin LD. Three-dimensional ultrastructural analysis of RNA distribution within germinal granules of *Xenopus*. Dev Biol. 2002; 241(1):79–93. [PubMed: 11784096]

Kloc M, Etkin LD. Delocalization of *vg1* mRNA from the vegetal cortex in *Xenopus* oocytes after destruction of *xlsirt* RNA. Science. 1994; 265:1101–1103. [PubMed: 7520603]

Kloc M, Etkin L. Two distinct pathways for the localization of RNAs at the vegetal cortex in *Xenopus* oocytes. Development. 1995; 121:287–297. [PubMed: 7539356]

Kloc M, Foreman V, Reddy SA. Binary function of mRNA. Review Biochimie. 2011b; 93(11):1955–1961. [PubMed: 21784124]

Kloc M, Larabell C, Chan AP-Y, Etkin LD. Contribution of METRO pathway localized molecules to the organization of the germ cell lineage. Mech Dev. 1998; 75:81–93. [PubMed: 9739112]

Kloc M, Spohr G, Etkin LD. Translocation of repetitive RNA sequences with the germ plasm in *Xenopus* oocytes. Science. 1993; 262:1712–1714. [PubMed: 7505061]

Kloc M, Wilk K, Vargas D, Shirato Y, Bilinski S, Etkin LD. Potential structural role of non-coding and coding RNAs in the organization of the cytoskeleton at the vegetal cortex of *Xenopus* oocytes. Development. 2005; 132(15):3445–3457. Erratum in: Development 132(24):5613. [PubMed: 16000384]

Koebernick K, Loeber J, Arthur PK, Tarbashevich K, Pieler T. Elr-type proteins protect *Xenopus dead end* mRNA from miR-18-mediated clearance in the soma. Proc Natl Acad Sci USA. 2010; 107(37):16148–16153. [PubMed: 20805475]

Kress TL, Yoon YJ, Mowry KL. Nuclear RNP complex assembly initiates cytoplasmic RNA localization. J Cell Biol. 2004; 165(2):203–211. [PubMed: 15096527]

Krieg PA, Melton DA. Developmental regulation of a gastrula-specific gene injected into fertilized *Xenopus* eggs. EMBO J. 1987; 4:3463–3471.

Ku M, Melton DA. Xwnt-11: a maternally expressed *Xenopus wnt* gene. Development. 1993; 119:1161–1173. [PubMed: 8306880]

Lewis RA, Gagnon JA, Mowry KL. PTB/hnRNPI is required for RNP remodeling during RNA localization in *Xenopus* oocytes. Mol Cell Biol. 2008; 28(2):678–686. [PubMed: 18039852]

Lustig KD, Kroll KL, Sun E, Kirschner MW. Expression cloning of a *Xenopus* T-related gene (*xombi*) involved in mesodermal patterning and blastopore lip formation. Development. 1996; 122:4001–4012. [PubMed: 9012520]

MacArthur H, Bubunenko M, Houston DW, King ML. *xcat2* RNA is a translationally sequestered germ plasm component in *Xenopus*. Mech Dev. 1999; 84:75–88. [PubMed: 10473122]

MacArthur H, Houston D, Bubunenko M, Mosquera L, King ML. DeadSouth is a germ plasm specific DEAD-Box RNA helicase in *Xenopus* related to eIF4A. Mech Dev. 2000; 95:291–295. [PubMed: 10906480]

Machado RJ, Moore W, Hames R, Houliston E, Chang P, King ML, Woodland HR. *Xenopus* Xpat protein is a major component of germ plasm and may function in its organization and positioning. Dev Biol. 2005; 287:289–300. [PubMed: 16216237]

Marlow FL, Yabe T, Adler A, Mullins MC, Pelegri F. Hecate/Grip2a acts to reorganize the cytoskeleton in the symmetry-breaking event of embryonic axis induction. PLoS Genet. 2014; 10(6):e1004422. Erratum in: PLoS Genet 10(10):e1004786. [PubMed: 24967891]

McMahon AP, Moon RT. Ectopic expression of the proto- oncogene *int1* in *Xenopus* embryos leads to duplication of the embryonic axis. Cell. 1989; 58:1075–1084. [PubMed: 2673541]

Melton DA. Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus* oocytes. Nature. 1987; 328:80–82. [PubMed: 3600777]

Messitt TJ, Gagnon JA, Kreiling JA, Pratt CA, Yoon YJ, Mowry KL. Multiple kinesin motors coordinate cytoplasmic RNA transport on a subpopulation of microtubules in *Xenopus* oocytes. Dev Cell. 2008; 15(3):426–436. [PubMed: 18771961]

Mosquera L, Forristall C, Zhou Y, King ML. A mRNA localized to the vegetal cortex of *Xenopus* oocytes encodes a protein with a nanos-like zinc finger domain. Development. 1993; 117:377–387. [PubMed: 8223259]

Mowry KL, Melton DA. Vegetal messenger RNA localization direted by a 340-nt RNA sequence element in *Xenopus* oocytes. Science. 1992; 255:991–994. [PubMed: 1546297]

Nakayama T, Fish MB, Fisher M, Oomen-Hajamos J, Thomsen GH, Grainger RM. Simple and efficient CRISPR/Cas9-mediated targeted mutagenesis in *Xenopus tropicalis*. Genesis. 2013; 51:835–843. [PubMed: 24123613]

Newport J, Kirschner M. A major developmental transition in early *Xenopus* embryos: I. characterization and timing of cellular changes at the midblastula stage. Cell. 1982; 30:675–686. [PubMed: 6183003]

Nieuwkoop PD. Origin and establishment of embryonic polar axes in amphibian development. Curr Top Dev Biol. 1977; 11:115–132. [PubMed: 332452]

Noro CY, Wylie C. Overexpression of cadherins and underexpression of β-catenin inhibit dorsal mesoderm induction in early *Xenopus* embryos. Cell. 1994; 79:791–803. [PubMed: 7528101]

Owens D, Butler A, Newman K, Aguero T, Van Booven D, King ML. RNA-Seq analysis of oocyte RNAs reveals novel regulatory pathways at the vegetal pole. Development. 2017 Accepted.

Pondel MD, King ML. A localized maternal mRNA related to TGF-β mRNA is concentrated in a cytokeratin enriched fraction from *Xenopus* oocytes. Proc Natl Acad Sci USA. 1988; 85:7612–7616. [PubMed: 2459710]

Ray D, Kazan H, Cook KB, Weirach MT, Najafabadi HS, Li X, Gueroussov S, Albu M, Zheng H, Yang A, Na H, Irimia M, Matzat LH, Dale RK, Smith SA, Yaroush CA, Kelly SM, Nabet B, Mecenas D, Li W, Laishram RS, Qiao M, Lipshitz HD, Piano F, Corbett AH, Carstens RP, Frey BJ, Anderson RA, Lynch KW, Penalva LO, Lei EP, Fraser AG, Blencowe BJ, Morris QD, Hughes TR. A compendium of RNA-binding motifs for decoding gene regulation. Nature. 2013; 499:172–177. [PubMed: 23846655]

Rebagliati MR, Weeks DL, Harvey RP, Melton DA. Identification and cloning of localized maternal RNAs from *Xenopus* eggs. Cell. 1985; 42:769–777. [PubMed: 2414011]

Richter JD, Smith LD, Anderson DM, Davidson EH. Interspersed poly(A) RNAs of amphibian oocytes are not translatable. J Mol Biol. 1984; 173:227–241. [PubMed: 6200602]

Rowning BA, Wells J, Wu M, Gerhart JC, Moon RT, Larabell CA. Microtubule-mediated transport of organelles and localization of β-catenin to the future dorsal side of *Xenopus* eggs. Proc Natl Acad Sci USA. 1993; 94:1224–1229.

Scharf SR, Gerhart J. Determination of the dorsal-ventral axis in eggs of *Xenopus laevis*: Complete rescue of uv-impaired eggs by oblique orientation before first cleavage. Dev Biol. 1980; 79:181–198. [PubMed: 7409319]

Schroder GF, Levitt M, Brunger AT. Super-resolution biomolecular crystallography with low-resolution data. Nature. 2010; 464:1218–1224. [PubMed: 20376006]

Smith LD. The role of a "germinal plasm" in the formation of primordial germ cells in *Rana pipiens*. Dev Biol. 1966; 14:330–347. [PubMed: 6008350]

Smith WC, Harland RM. Injected *xwnt-8* RNA acts early in *Xenopus* embryos to promote formation of a vegetal dorsalizing center. Cell. 1991; 67:753–765. [PubMed: 1657405]

Sokol S, Christian JL, Moon RT, Melton DA. Injected *wnt* RNA induces a complete body axis in *Xenopus* embryos. Cell. 1991; 67:741–752. [PubMed: 1834344]

Stennard F, Carnac G, Gurdon JB. The *Xenopus* T box gene *antipodean* encodes a vegetally localized maternal mRNA that can trigger mesoderm formation. Development. 1996; 122:4179–4188. [PubMed: 9012537]

Summerton J, Weller D. Morpholino antisense oligomers: Design, preparation and properties. Antisense & Nucleic Acid Drug Development. 1997; (3):187–195. [PubMed: 9212909]

Tan HL, Queenan BN, Huganir RL. GRIP1 is required for homeostatic regulation of AMPAR trafficking. Proc Natl Acad Sci U S A. 2015; 112(32):10026–10031. [PubMed: 26216979]

Tao Q, Yokota C, Puck H, Kofron M, Birsoy B, Yan D, Asashima M, Wylie CC, Lin X, Heasman J. Maternal *wnt11* activates the canonical wnt signaling pathway required for axis formation in *Xenopus* embryos. Cell. 2005; 120(6):857–871. [PubMed: 15797385]

Tarbashevich K, Koebernick K, Pieler T. XGRIP2.1 is encoded by a vegetally localizing, maternal mRNA and functions in germ cell development and anteroposterior PGC positioning in *Xenopus laevis*. Dev Biol. 2007; 311(2):554–565. [PubMed: 17936745]

Torpey N, Wylie CC, Heasman J. Function of maternal cytokeratin in *Xenopus* development. Nature. 1992; 357:413–415. [PubMed: 1375708]

Venkataraman, T., Dancausse, E., King, ML. "PCR-based cloning and differential screening of RNAs from *Xenopus* primordial germ cells: Cloning uniquely expressed RNAs from rare cells. In: Schatten, H., editor. Methods in Molecular Biology, Germ Cell Protocols Vol 2: Molecular Embryo Analysis, Live Imaging, Transgenesis and Cloning. Totowa, NJ: Humana Press Inc; 2004.

Wang H-W, Fang J-S, Kuang X, Miao L-Y, Xia G-L, King ML, Zhang J. Long-chain acyl-CoA synthetase 1 is required for maintaining meiotic arrest in *Xenopus* and mouse oocytes. Biol of Reproduction. 2012; 87:1–9.

Weaver C, Kimelman D. Move it or lose it: axis specification in Xenopus. Development. 2004; 131:3491–3499. [PubMed: 15262887]

Weeks DL, Melton DA. A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGF-beta. Cell. 1987; 51:861–867. [PubMed: 3479264]

Wilson, EB. The Cell in Development and Heredity. 3rd. New York: The MacMillan Company; 1925.

Woodland HR, Gurdon JB, Lingrel JB. The translation of mammalian globin mRNA injected into fertilized eggs of *Xenopus laevis*. II. The distribution of globin synthesis in different tissues. Dev Biol. 1974; 39:134–140. [PubMed: 4857973]

Yisraeli JK, Melton DA. The material mRNA *vg1* is correctly localized following injection into *Xenopus* oocytes. Nature. 1988; 336:592–595. [PubMed: 3200307]

Yisraeli JK, Sokol S, Melton DA. A two-step model for the localization of maternal mRNA in *Xenopus* oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of *vg1* mRNA. Development. 1990; 108:289–298. [PubMed: 2351071]

Zearfoss NR, Chan AP, Wu CF, Kloc M, Etkin LD. Hermes is a localized factor regulating cleavage of vegetal blastomeres in *Xenopus laevis*. Dev Biol. 2004; 267(1):60–71. [PubMed: 14975717]

Zhang J, Houston D, King ML, Payne C, Wylie C, Heasman J. The role of maternal VegT in establishing the primary germ layers in Xenopus embryos. Cell. 1998; 94:515–524. [PubMed: 9727494]

Zhang J, King ML. *Xenopus vegT* RNA is localized to the vegetal cortex during oogenesis and encodes a novel T-box transcription factor involved in mesodermal patterning. Development. 1996; 122:4119–4129. [PubMed: 9012531]

Zhou Y, King ML. Localization of *xcat-2* RNA, a putative germ plasm component, to the mitochondrial cloud in *Xenopus* stage I oocytes. Development. 1996a; 122:2947–2953. [PubMed: 8787767]

Zhou Y, King ML. RNA transport to the vegetal cortex of *Xenopus* oocytes. Dev Biol. 1996b; 179:173–183. [PubMed: 8873762]

Zhou Y, Zhang J, King ML. *Xenopus* ARH couples lipoprotein receptors with the AP-2 complex in oocytes and embryos and is required for vitellogenesis. J Biol Chem. 2003; 278:44584–44592. [PubMed: 12944396]

Zhou Y, Zhang J, King ML. Polarized distribution of mRNAs encoding a putative LDL receptor adaptor protein, xARH (Autosomal Recessive Hypercholesterolemia) in *Xenopus* oocytes. Mech Dev. 2004; 121:1249–1258. [PubMed: 15327785]

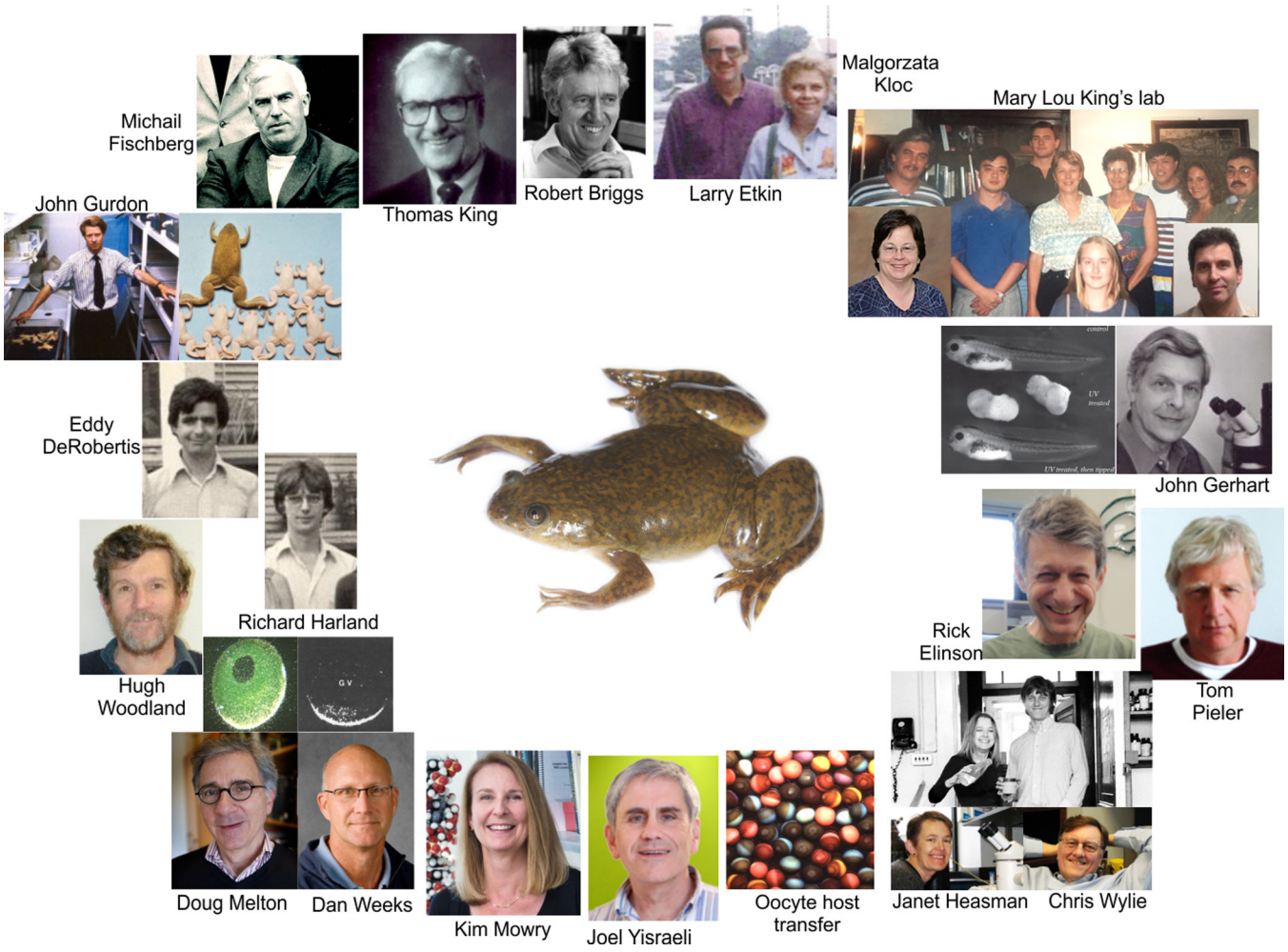

Figure 1.