

Spatiotemporal Changes in Metallothionein Gene Expression During Embryogenesis in the Wall Lizard *Podarcis sicula*

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ABSTRACT

Lizard embryos are nutritionally independent from their environment. During the early phases of oogenesis, the egg prepares for development by storing reserve organelles, proteins, and RNAs sufficient to allow the zygote to transform into a juvenile. This preparation also includes the storage of metallothionein (MT) transcripts. This study investigated the localization of these transcripts by in situ hybridization throughout *Podarcis sicula* developmental stages. Our data show that MT expression undergoes shifts in both regional and cellular localization. MT transcripts were detected early in the central nervous system, later in tissues implicated in metabolic processes. Results are discussed highlighting differences in lizard embryonic spatial and temporal MT expression compared with piscine, amphibian, and mammalian embryos. We hypothesize that, under natural conditions, the nutritionally closed system represented by the lizard egg protects the developing embryo from an unwanted excess of metals. This mechanism would make MT expression and accumulation in detoxifying organs in developing animals unnecessary until hatching and food intake begins. Conversely, the presence of MT transcripts during brain development may ensure the correct final architecture of this organ. *J. Exp. Zool.* 313A:410–420, 2010. © 2010 Wiley-Liss, Inc.

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Metallothioneins (MTs), small cysteine-rich intracellular proteins that bind the essential heavy metals zinc and copper, are found in all animals so far examined (Palmiter, '98; Coyle et al., 2002). MTs are implicated in metal metabolism, cellular repair processes, growth, and differentiation (Vasák and Hasler, 2000; Dutsch-Wicherek et al., 2008). They likely serve as a zinc, and to a lesser extent, copper store for newly synthesized apoenzymes (Bell and Vallee, 2009). These metals are involved in various functions, including control of gene transcription, nerve conductance, oxygen transport, and as active centers in enzymes (Cai et al., 2005). Therefore, critical molecular events within the cell, such as gene expression, cell proliferation, and cell death, are affected by trace elements (Dreosti, 2001; Klaahsen et al., 2007). In addition, some toxic metals may mimic the essential metals and thereby gain access to important molecular targets (Waalkes, 2003; Rana,

2008). MTs, therefore, play a homeostatic role in the control and detoxification of the heavy metals (Carginale et al., '98; Roesijadi, 2000; Cobbett and Goldsbrough, 2002); they also have the capacity to scavenge reactive oxygen species, particularly the hydroxyl radical (Sato and Kondoh, 2002; Bell and Vallee, 2009).

MTs may also serve critical functions during embryonic development, as suggested by their expression in early

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embryogenesis of plant and animal species (Hamer, '86; Kawashima et al., '92). In vertebrate embryos, early transcription of MT genes has been observed in rainbow trout (Olsson et al., '90), zebrafish (Riggio et al., 2003a; Chen et al., 2004), *Xenopus* (Durliat et al., '99), birds (Richards, '84), and mammals, such as rabbit and mouse (Andrews et al., '87, '91, 2001). In mouse, MT mRNAs are present as maternal messengers stored in eggs; the MT-I and MT-II genes are among the first to be transcribed from the embryonic genome. They are coordinately expressed throughout the preimplantation period of embryonic development (Andrews et al., '91). These findings suggest a possible physiological role of MT during development. Nevertheless, describing a role for MT in vertebrate development has been elusive, because neither metabolic nor developmental effects are seen in MT gene knockout mice (Palmiter, '98).

Generally, mammalian embryos obtain nutrients continuously as they develop, and for this reason they are defined as "open systems" capable of exchanging metals and other micronutrients with the environment (Davidson, '90). On the contrary, eggs of oviparous vertebrates mature over a long period and contain all the resources needed for full embryonic development before fertilization (Thompson and Speake, 2003). Only the water content in reptilian eggs is insufficient and the water exchange through the parchment shell is necessary for a successful embryonic development (Belinsky et al., 2004). Therefore, embryos of oviparous vertebrates are considered "closed systems," independent of the environment as a source of nutrition (Nomizu et al., '93). Hence, during the early phases of oogenesis, a number of synthetic processes prepare for embryonic development by forming sufficient DNA, nucleoproteins, and mRNAs that allow the single cell to be transformed into a juvenile form. This preparation also includes the storage of vitellogenin and other molecules in yolk platelets (Motta et al., 2001; Tammaro et al., 2007).

In an earlier article, we demonstrated that MT transcripts are always present in oocytes and ovaries of the oviparous vertebrate *Podarcis sicula* (the Italian wall lizard), even in the absence of the protein (Riggio et al., 2003b). In all the tissues examined, the transcripts encode the same MT isoform (Trinchella et al., 2006, 2008). This MT binds copper, zinc, and, in case of contamination, cadmium. The protein is constitutively present in the liver, whereas in the ovary, the synthesis is observed only in cadmium-treated lizards (Riggio et al., 2003b), thus indicating for this MT a dual role in the essential metal ions homeostasis and in detoxification against non-essential metals.

The lizard MT-mRNA content is very low in small previtellogenic oocytes, but increases greatly in large vitellogenic oocytes and ovulated eggs. Similarly, in the ovary, the highest MT-mRNA level is detected during the reproductive period when the ovary contains large vitellogenic follicles and the ovulation occurs (Riggio et al., 2003b). This evidence suggests that the MT-mRNA storage in *Podarcis* eggs occurs possibly to meet the future needs of the growing embryo.

In this work, we investigated the spatial and temporal localization of MT transcripts by in situ hybridization throughout *P. sicula* development, to determine if the expression pattern in this oviparous nutritionally "closed system" is similar to that of the mammalian "open system."

MATERIALS AND METHODS

Adult Animals

Sexually mature females of *P. sicula* were captured on the outskirts of Naples, and maintained in an animal house under a natural photothermal regime, and fed ad libitum. The ovaries were removed during the reproductive period and processed for cytological and biomolecular investigation.

All experiments were organized to minimize stress in the number of animals used and were performed in accordance with the Guideline for Animal Experimentation of the Italian Department of Health, under the supervision of a veterinarian.

Embryos

Embryos were obtained from gravid females in the spring between May and June. Eggs collected from a single clutch were developed in a terrarium maintained at natural temperature (range 20–25°C); water lost as vapor was reintroduced by daily soil nebulizations with tap water.

The embryos were collected from preoviposition eggs at regular time intervals (5, 10, 20, 40 and 50 days), from deposition until hatching. After opening the egg envelopes, embryos were recovered from shells, photographed, and immediately processed for cytological or biomolecular investigations. Embryo staging was based on the developmental tables of *Lacerta vivipara* (Dufaure and Hubert, '61).

Light Microscopy

Ovaries and embryos were fixed in Bouin's solution and processed for paraffin wax embedding according to routine protocols. Sections were stained with haematoxylin-eosin or Mallory's trichrome to show general morphology.

MT mRNA Detection by In Situ Hybridization

Sections (5–7 µm) were placed on superfrost glass slides (Menzel-Glaser, Germany), fixed in paraformaldehyde 4% PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄) pH 7.4 for 20 min, and incubated in PK buffer (Tris-HCl, 0.2 M, pH 7.4, EDTA 0.01 M, pH 8, proteinase K, 10 µg/mL, H₂O_{dep}) at 37°C for 15 min. After washing in PBS, they were incubated at 42°C for 90 min in a prehybridization mix containing formamide, SSC 4 × and 1 × Denhart's solution. Hybridization was carried out at 42°C overnight using a dig-labeled cDNA probe encoding the *P. sicula* MT (Trinchella et al., 2006). Sections were washed in SSC 2 × (Tris-HCl 0.1 M, pH 7.5, NaCl 0.1 M, H₂O_{dep}) and in Buffer I containing the Blocking reagent (0.5%). Digoxigenin was

revealed by incubating sections with an AP-conjugated anti-dig antibody diluted 1:400 overnight. Slides were washed in Buffer I, incubated with levamisole-Tween20 $1 \times$ for 15 min and revealed with BM-Purple.

Dig-labeled MT cDNA probe was generated by PCR using the DIG High Prime DNA labeling and detection starter kit I (Roche Diagnostics, Germany).

In the negative control, the hybridization solution did not contain MT cDNA probe.

RESULTS

MT mRNA Distribution in *P. sicula* Ovarian Follicles

In early 80–150 μm diameter previtellogenic follicles, MT transcripts were localized in the cytoplasm and, to a lesser extent, in the nucleus of oocytes, whereas the small stem cells forming the follicular epithelium were unlabeled (Fig. 1A, B). In 400–1,400 μm mid-previtellogenesis follicles, an intense hybridization signal was present in the epithelium, in particular, in the cytoplasm of both small stem cells and differentiated pyriforms (Fig. 1C–E). In larger previtellogenic follicles (1,500–2,000 μm), the MT-mRNA remained localized in small cells but was

significantly reduced in pyriforms (Fig. 1F). In vitellogenic follicles of 2,000 μm or larger, MT-mRNA was present in the cytoplasm of all small cells (Fig. 1G). Controls prepared omitting the probe in the hybridization mixture showed completely unstained follicles (Fig. 1H).

MT mRNA Distribution in *P. sicula* Developing Embryos

Embryos at Discoblastula Stage. Embryos at this stage were collected from preoviposition eggs. The slides were prepared by squashing the animal pole of the embryos between the slide and cover slip. An intense hybridization signal was present in the cytoplasm of both embryonic (Fig. 2A–C) and extraembryonic (Fig. 2D) blastomeres.

Embryos at 5 Days from Ovodeposition (Stage 29). At this stage (Fig. 3A), embryos show a well-defined head and a prominent brain with expanded optic vesicles. The pharyngeal pouches (branchial slits) are recognizable in the neck region and in the trunk; the limb buds are already formed.

The in situ hybridizations reveal that MT transcripts are present in the ventricular zone of the developing brain vesicles (Fig. 3B, C) and neural tube (Fig. 3D, E); the marginal zone is

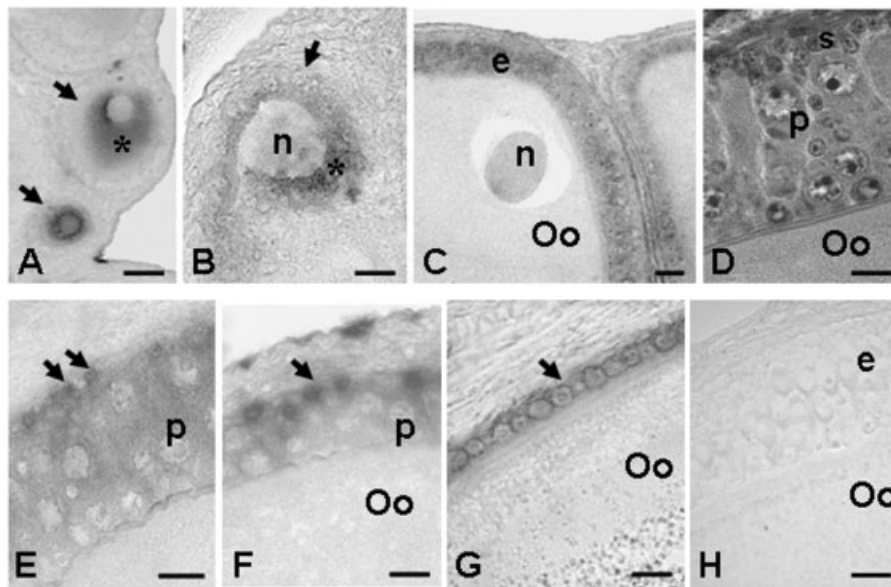


Figure 1. MT-mRNA localization in the ovarian follicles of adult lizards. (A) Early previtellogenic oocytes (arrows) with stained cytoplasm (*). (B) Details showing the weak-labeled oocyte nucleus (n) and the intensely labeled oocyte cytoplasm (*). Follicle cells (arrow) are unlabeled. (C) Mid previtellogenic follicles with labeled epithelia (e). Note the weak labeling on the oocyte (Oo) nucleus (n). (D) Details of a follicular epithelium stained with Mallory's trichrome. Note the small cells (s) and the pyriforms (p). (E) Same epithelium showing messenger in the cytoplasm of small cells (arrows) and pyriforms (p). (F) Late previtellogenic follicle. MT-mRNA is abundant in small cells (arrow) while pyriforms (p) appear almost unstained. (G) Epithelium in an early vitellogenic follicle. Messenger is present in the cytoplasm of all the small cells (arrow). (H) Unstained negative control prepared omitting the probe from the hybridization mixture. Oo, Oocyte; e, epithelium. Bars: (A) 50 μm ; (B–F) 20 μm ; (G, H) 10 μm .

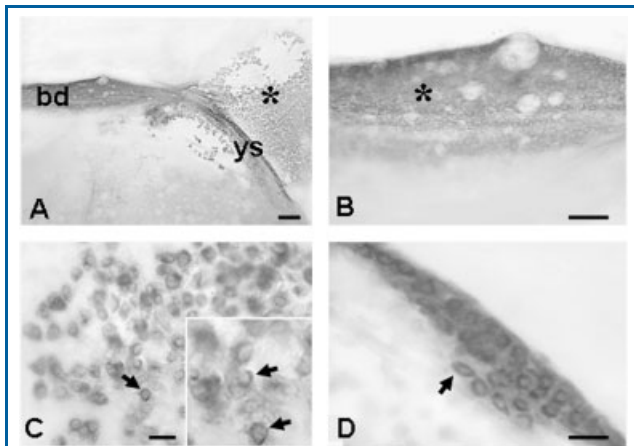


Figure 2. MT-mRNA localization in embryos at the discoblastula stage. (A) Blastodisc (bd) and yolk sac (ys) with dispersed cells (*). (B) Details showing labeling distribution in the blastodisc cells (*). (C) Dispersed embryo cells with labeled cytoplasm (arrows). Insert: detail. (D) Yolk sac cells with intensely labeled cytoplasm (arrow). Bars: (A) 100 μm ; (B) 50 μm ; (C) 30 μm ; (D) 15 μm .

completely unstained. MT mRNA is also present in the eye, the undifferentiated retina (Fig. 3F) and in the lens epithelium (Fig. 3F; inset), in mesenchymal cells, and in somites (Fig. 3G). No hybridization signal is observed in kidney, gut, liver (Fig. 3H, I), and lung (data not shown).

Embryos at 10 Days from Ovodeposition (Stage 31). In these embryos, the large mesencephalon protrudes dorsally from the cranial vault, the eyes are also prominent and slightly pigmented, and the limbs are paddle shaped with clearly recognizable stylopode and zeugopode. The tail is longer and thinner than in the earlier stage; two branchial slits are still visible (Fig. 4A).

The *in situ* hybridizations reveal the absence of significant changes in MT mRNA localization with respect to stage 29 embryos. In brain vesicles (Fig. 4B–D) and in the spinal cord (Fig. 4E), transcripts are localized only in the ventricular zones. MT mRNA is also present in the undifferentiated retina (Fig. 4B) and in somites (Fig. 4F), but absent in kidney, lung, gut, and liver (Fig. 4G–J).

Embryos at 20 Days from Ovodeposition (Stage 33). At this time, the optic lobes of the mesencephalon are very prominent, the eye is pigmented, the lens differentiated, and a circular eyelid begins to develop; the limb paddle contains three distinct fingers (Fig. 5A).

The MT-mRNA distribution slightly changes compared with the earlier stage because the ventricular zones are reduced while the marginal zones expand and differentiate, as neurons arranged in distinct nuclei. In the telencephalon (Fig. 5B), diencephalon, and spinal cord (data not shown), labeling remains exclusively on the ventricular zones while in the mesencephalon

(Fig. 5C) and in the medulla oblongata (data not shown), labeling also appears on the developing optic cortex and on several nuclei. In all vesicles, significant labeling also appears on the ependymal cells (Fig. 5C).

In the eye, the retinal stratification (Fig. 5D) results in a redistribution of MT-mRNA that concentrates in the ganglion cells layer and in the inner and outer nuclear layers (Fig. 5E). In the trunk, an intense hybridization signal is present in the differentiating vertebrae (Fig. 5F). Of significant novelty is the appearance of a positive signal in renal glomeruli (Fig. 5G), whereas the tubules (Fig. 5G), lung, liver (Fig. 5H), and the gut mucosa (Fig. 5I) remain unstained.

Embryos at 40 Days from Ovodeposition (Stage 38). At this stage (Fig. 6A), the mesencephalic lobes are less pronounced while the telencephalon becomes evident. The eyes are also less prominent than in the earlier stages and show completely developed eyelids. In the limbs, digits are fully separated.

The *in situ* hybridizations demonstrate that significant changes have occurred in MT-mRNA expression. Messengers are no longer present in the telencephalon (Fig. 6B), diencephalon, and mesencephalon (Fig. 6C), whereas in the oblongata (Fig. 6D) and cerebellum (Fig. 6C), they are concentrated in the nuclei, and granular and molecular layers, respectively. In the spinal cord (Fig. 6E, F), messengers are present in the white matter, and in both fibers and nuclei of glial cells, whereas the grey matter is completely unlabeled.

In the differentiated retina (Fig. 6G), messengers are in the inner and outer nuclear layers but not in the ganglion cells layer (Fig. 6H). In visceral organs, the MT-mRNA appears in the lung parenchyma (Fig. 6I), but not in the liver (Fig. 6I), gut mucosa (Fig. 6J), and kidney tubules (data not shown), which remain unstained.

Pre-hatching Embryos. Fifty-five days after ovodeposition (Fig. 7A), the embryos have acquired their typical skin pigmentation and have differentiated scales over the body; nails are also distinctly visible. Hatching occurred between day-58 and 60 post-deposition.

At this stage, the distribution of MT transcripts undergoes further relevant changes. Significant labeling appears on the cortical areas of the telencephalon (Fig. 7B), ependymal cells of telencephalon (Fig. 7B, C), diencephalon (Fig. 7D), and mesencephalon (Fig. 7E), optic cortex, the commissura ansulata, and several basal nuclei. MT-mRNA distribution in the medulla oblongata and spinal cord does not change with respect to the earlier stage (data not shown).

In the differentiated retina (Fig. 7F), the MT-mRNA is present in the outer and inner nuclear layers and also in the ganglion cells layer. The two plexiform layers and optic fibers layer are unstained.

In the visceral organs, a significant hybridization signal is observed for the first time on the liver, cytoplasm of the Kupffer

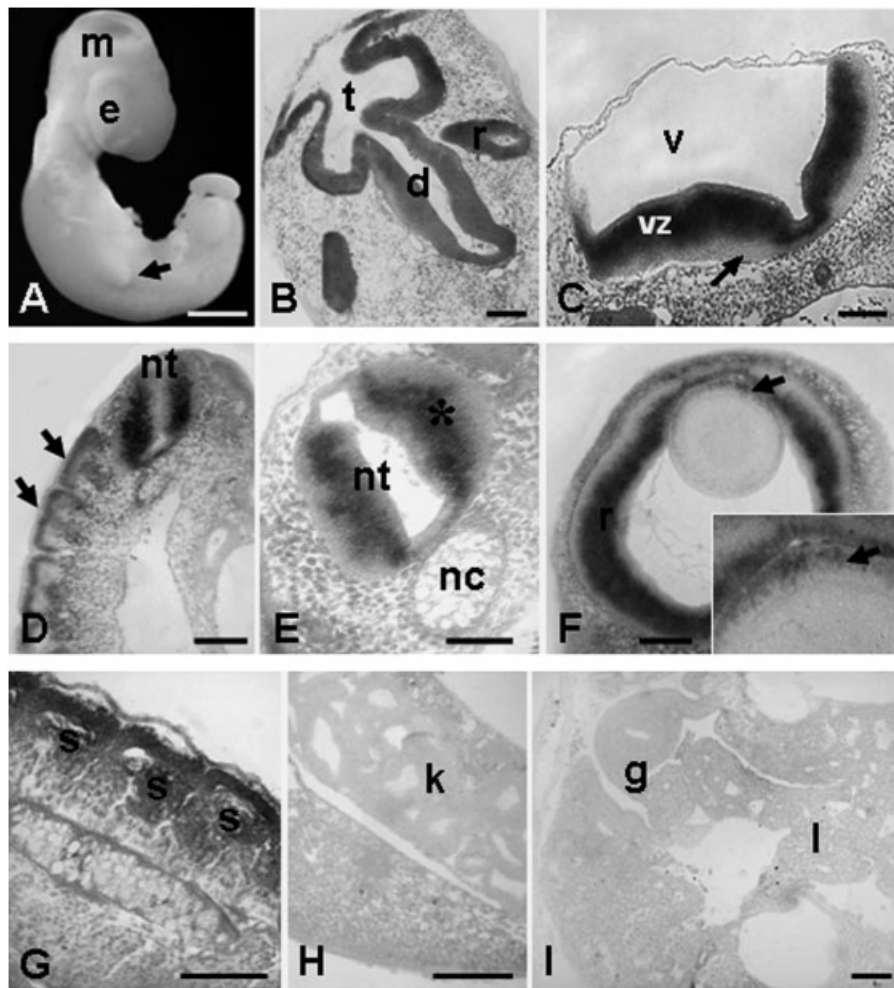


Figure 3. MT-mRNA localization in embryos 5 days post-deposition (stage 29). (A) In toto embryo with prominent eye (e) and mesencephalon (m). Limb bud (arrow). (B) Frontal section of the head; undifferentiated diencephalic (d) and telencephalic (t) vesicles with uniformly labeled walls. Retina (r). (C) Cross-section of medulla oblongata. MT-mRNA is concentrated in the ventricular zone (vz), the marginal zone is unstained (arrow). Fourth ventricle (v). (D) Oblique section of the tail showing the labeled neural tube (nt) and somites (arrows). (E) Detail of the neural tube (nt) with messenger concentrated in the ventricular zone (*). Notochord (nc). (F) Eye with abundant MT-mRNA in the undifferentiated retina (r) and lens epithelium (arrow and inset). (G) Details of labeled somites (s); (H, I) unlabelled kidney (k), gut (g) and liver (l). Bars: (A) 400 μm ; (B–I) 100 μm .

cells and monocytes (Fig. 7G, H), and gut mucosal cells (Fig. 7I). No changes are observed in the lung parenchyma (Fig. 7J), which remains stained, and kidney tubules (data not shown) that remain unstained.

DISCUSSION

Telolecitic eggs, typical of oviparous vertebrates, contain all the resources needed to sustain the coordinated steps leading to differentiation, migration, and functional organ formation. In the lizard *P. sicula*, MT transcripts accumulate as part of these

resources, possibly to cope with the future needs of the growing embryo (Riggio et al., 2003b).

Here, we demonstrate that MT transcription is initially carried out directly by the oocyte; later, this synthesis occurs in the pyriforms, the nurse cells known to transfer organelles and RNAs to the oocyte via intercellular bridges (Taddei, '72; Andreuccetti et al., '78; Motta et al., '95, '96).

The MT-mRNA accumulation during oogenesis has also been demonstrated in other species. In several cases, such as in *Danio* (Chen et al., 2004), high levels of MT transcripts are accompanied by high levels of protein (Riggio et al., 2003a). In other cases, for

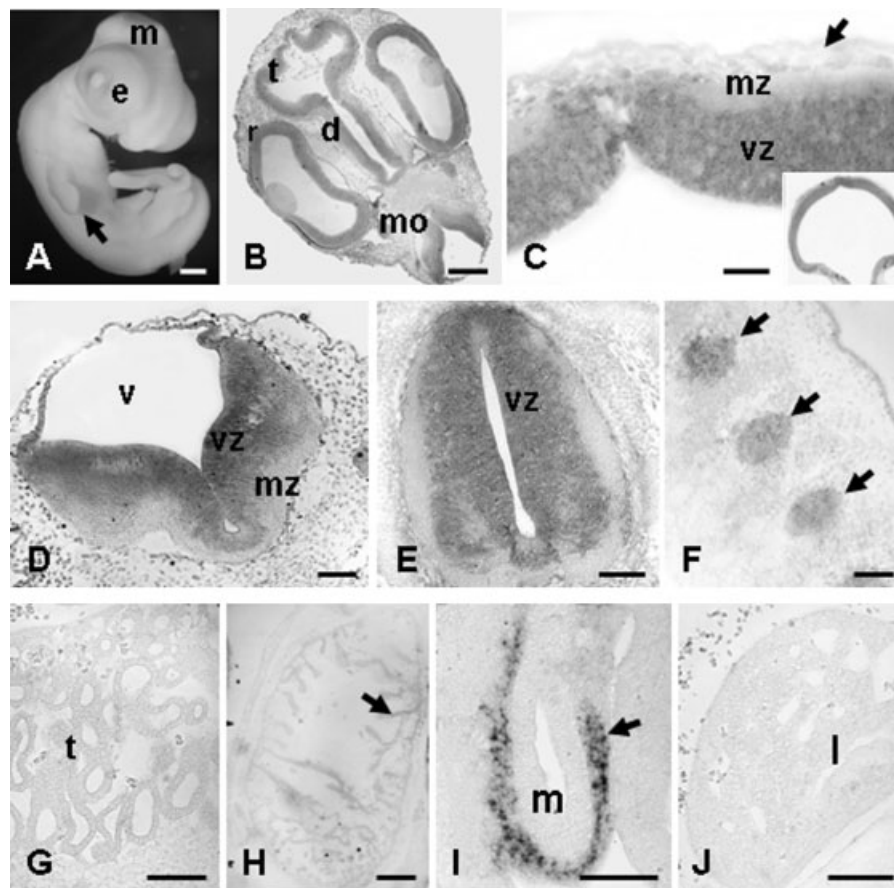


Figure 4. MT-mRNA localization in embryos 10 days post-deposition (stage 31). (A) In toto embryo with pigmented eye (e), protruding mesencephalon (m), and paddle-shaped limbs (arrow). (B) Frontal section of the head. Telencephalon (t), diencephalon (d), medulla oblongata (mo), and retina (r) show significant and uniform labeling; the lens is unstained. (C) Details of the optic tectum; labeled ventricular zone (vz), unlabeled marginal zone (mz), and meningeal layer (arrow). Inset: low magnification of the developing tectum. (D) Cross-section of the medulla oblongata; no significant difference with respect to stage 29 can be observed. IV ventricle (v), marginal (mz), and ventricular (vz) zones. (E) Cross-section of the neural tube; mRNA localized in ventricular zone (vz). (F) Labeled somites (arrows). (G) Unlabeled kidney tubules (t). (H) Unlabeled lung septa (arrow). (I) Unlabeled intestinal mucosa (m) surrounded by labeled connective cells (arrow). (J) Unlabeled liver parenchyma (l). Bars: (A) 0.5 mm; (B) 300 μ m; (C–E and G–J) 50 μ m; (F) 100 μ m.

example, the rainbow trout, MT-mRNA sharply declines during oogenesis so that the eggs contain neither messenger nor protein (Olsson et al., '90).

In situ hybridizations have clarified the precise tissue distribution of MT transcripts during lizard development. In the early phase of development, at the late blastula stage, transcripts are present in the cytoplasm of both embryonic and extra embryonic cells. Currently, we do not know whether this could be ascribed to a uniform distribution of earlier accumulated maternal mRNAs or to de novo synthesis. The expression of the same MT isoform in embryonic and adult tissues does not help us to discriminate between the maternal and embryonic transcripts.

The presence of MT-mRNA in vertebrate early embryogenesis is quite variable among different species. *Podarcis* more closely resembles mammals than other lower vertebrates studied so far. In mammals, MT genes are among the first to be activated in the embryo genome (Andrews et al., '91), whereas in *Xenopus* (Durliat et al., '99) or trout (Olsson et al., '90), for example, MT-mRNA appears only after the early tailbud or the gastrula stages, respectively.

At the beginning of organogenesis in *Podarcis*, MT-mRNA distribution changes. Specifically, it concentrates in the neural tube and retina, mesodermal derivatives, such as somites and mesenchyme, but not in kidney tubules or in the main

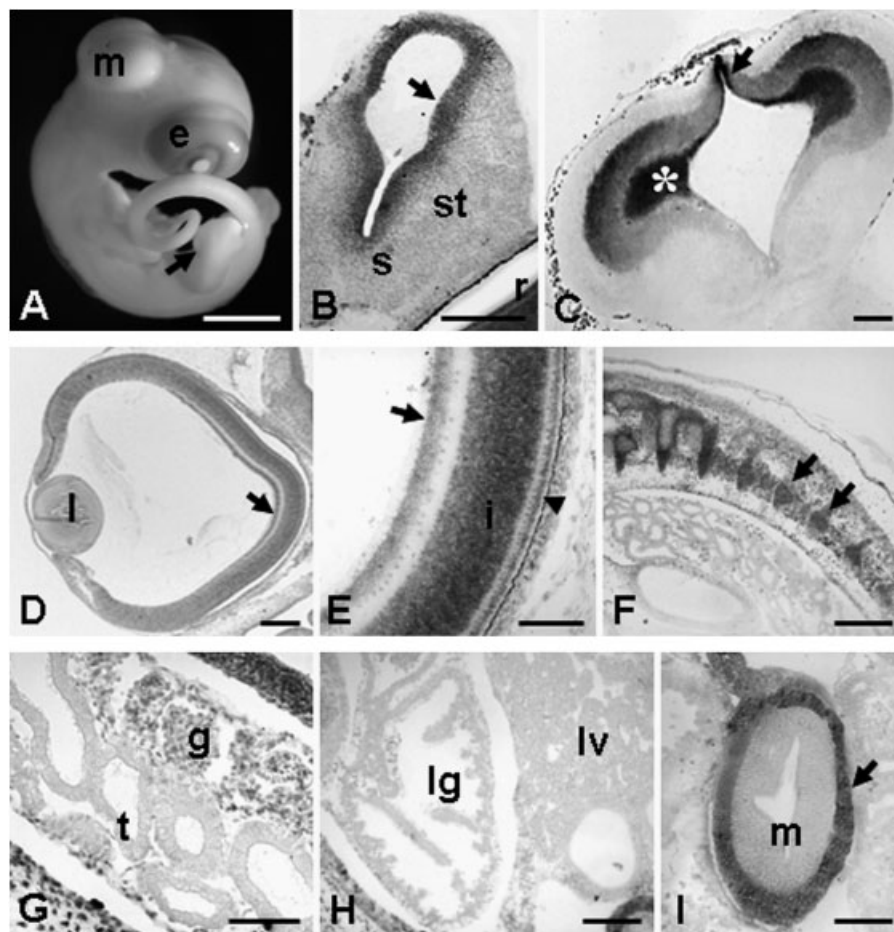


Figure 5. MT-mRNA localization in 20-day old embryos (stage 33). (A) In toto embryo with prominent midbrain (m) and pigmented eye (e). The limb paddle shows three digits (arrow). (B) Cross-section of the telencephalic hemisphere. The MT-mRNA is localized in the ventricular zone (arrow). Septum (s), striatum (st), and retina (r). (C) Cross-section of the optic lobes; labeling is concentrated in the developing cortex (*). The dorsal ependymal cells are intensely labeled (arrow). (D) Eye, haematoxylin and eosin staining showing the stratified posterior retina (arrow); lens (l). (E) Details of the posterior retina showing labeling on the ganglion cells layer (arrow), the inner (i) and outer (arrowhead) nuclear layers. (F) Backbone with labeled vertebrae (arrows). (G) Sagittal section of kidney; notice the presence of MT-mRNA in the glomeruli (g) but not in the tubules (t). (H) Unlabeled lung septa (lg) and liver parenchyma (lv). (I) Unlabeled gut mucosa (m) surrounded by labeled connective cells (arrow). Bars: (A) 2 mm; (B–D, F) 100 μ m; (E, G–I) 50 μ m.

endodermal derivatives, such as gut mucosa and the anlagen of liver and lung.

The absence of MT transcripts in gut, liver, and kidney persists throughout the development. This may be owing to peculiarities of this “closed system,” where embryos grow using the internal egg nutrient content and detoxifying organs are not yet required. Immediately before hatching, MT transcripts appear and this may be related to the fact that the embryo prepares to become an “open system” obtaining food, in particular, from the environment.

The embryo, however, is prepared to cope with accidental or abnormal contact with heavy metals. Incubation of eggs in

cadmium-contaminated soil induces early MT-mRNA synthesis in liver (unpublished data). It is significant that activation occurs only in the Kupffer cells/monocytes, the non-parenchymal cells with detoxifying activity (Naito et al., 2004). These are the same cells responsible for cadmium detoxification in adult liver (Simoniello et al., 2010).

These preliminary data suggest that the intake of water carrying metallic contaminants can affect and change the expression of the MT embryonic gene. Indeed, in ovo cadmium exposure modifies the transcriptional regulation of gene expression in *P. sicula* embryos (Trinchella et al., 2010).

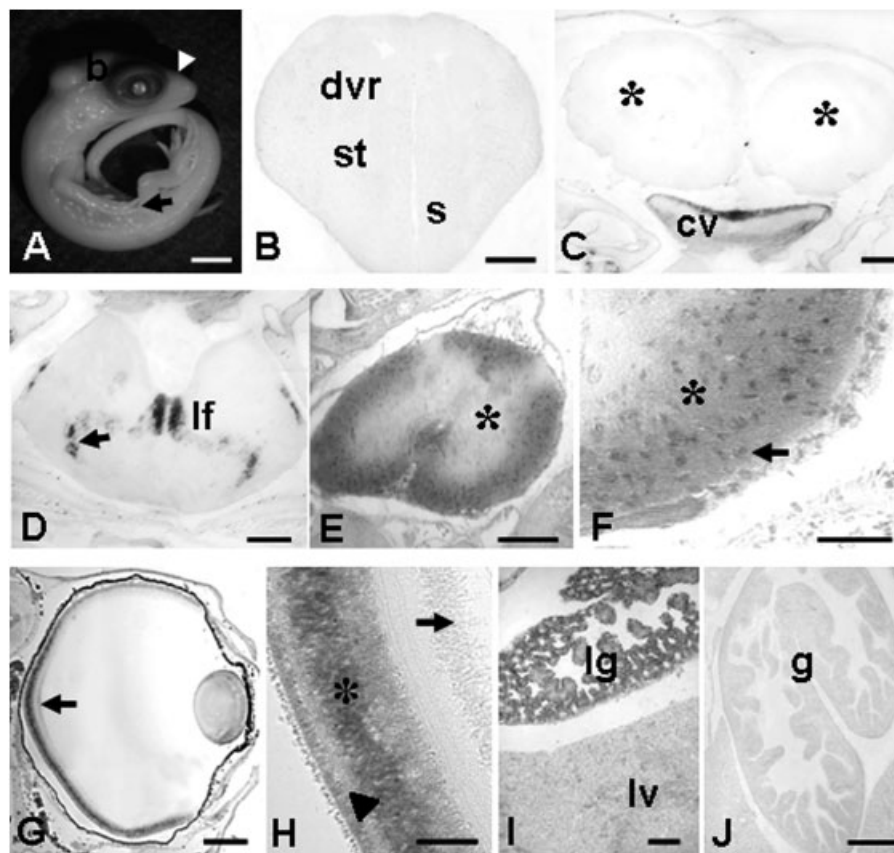


Figure 6. MT-mRNA localization in embryos 40 days post-deposition (stage 38). (A) In toto embryo with fully differentiated eyelid (arrowhead) and limbs (arrow). Brain (b). (B) Cross-section of the telencephalon with unlabeled septum (s), striatum (st), and dorsal ventricular ridge (dvr). (C) Cross-section, completely unlabeled mesencephalon (*). Note the labeled cerebellar cortex (cv). (D) Cross-section of the medulla oblongata with the labeled basal nuclei (arrow) and medial longitudinal fascicle (lf). (E) Cross-section of neural tube with unlabeled white matter (*). (F) Detail showing the presence of messenger in the axons (*) and nuclei of glial cells (arrow). (G) Eye; mRNA is concentrated in the retina (arrow). (H) Detail showing the stained inner (*) and outer (arrowhead) nuclear layers and the unstained ganglion cells layer (arrow). (I) Unlabeled liver (lv) and labeled lung (lg) parenchyma. (J) Unlabeled gut (g). Bars: (A) 2.5 mm; (B–D, G, J) 200 μ m; (E, I) 100 μ m; (F, H) 50 μ m.

Expression of the MT gene in the *Podarcis* central nervous system deserves special attention. Data clearly show that MT expression undergoes shifts in both regional and cellular localization during development. In early embryos, transcripts show a broad distribution in ventricular areas of the undifferentiated vesicles. In the following 20 days, MT-mRNA appears in the ependymal cells and developing grey matter, particularly the brain nuclei and cortices and in the horns of the neural tube.

Around day-40, transcripts peculiarly, but temporarily, disappear from the telencephalon, diencephalon, and mesencephalon but not from the posterior districts. These data are particularly striking when considering that all areas of the three vesicles become simultaneously unlabeled. It is of particular interest that MT-mRNA concomitantly disappears also from the

ganglion cell layer and grey matter of the neural tube. These events suggest either a consumption of the pool of maternal mRNAs or, alternatively, a temporary silencing of the MT embryo gene. What really happens and the nature of the triggering factors remains to be clarified.

Taken together, the data presented in this study show the complexity of the pattern of expression of MT genes during vertebrate development, and highlight differences in lizard embryonic spatial and temporal MT expression compared with piscine, amphibian, and mammalian embryos. In the latter, the expression of MT gene is activated early and abundantly in the visceral organs with detoxifying functions, whereas the gene is activated late, immediately before the birth or at birth, in the brain. In mammals, the expanded function of MTs in brain are a

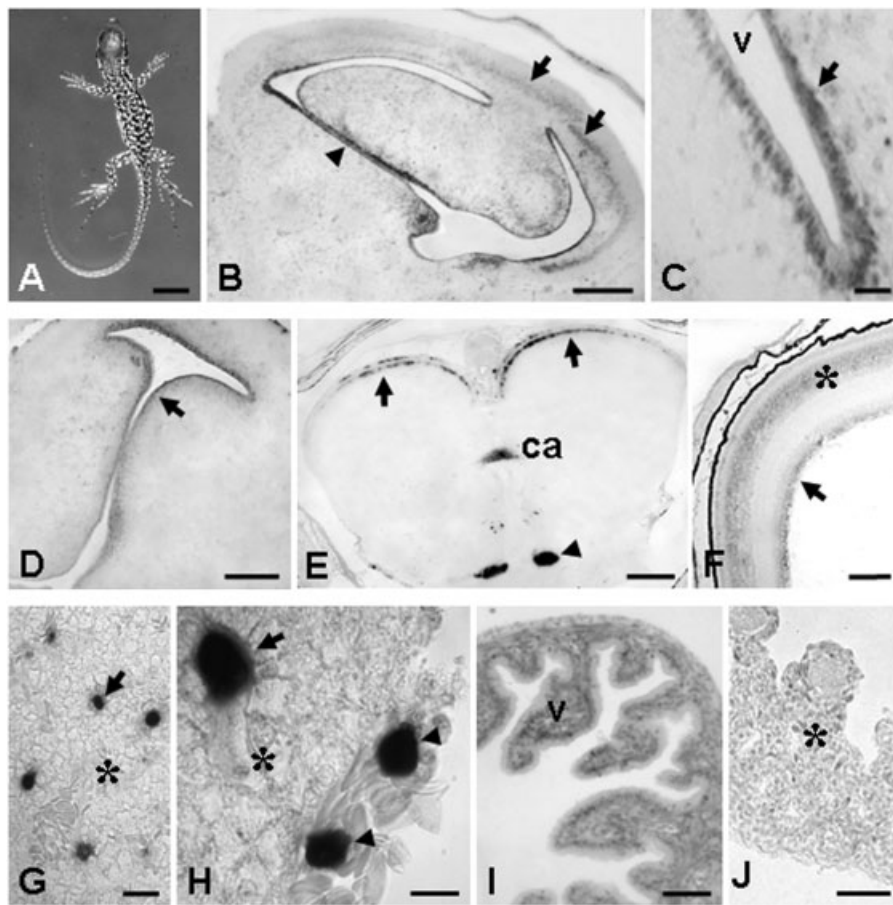


Figure 7. MT-mRNA localization in pre-hatching embryos. (A) Fully developed embryo; the skin is pigmented as in the adult. (B) Frontal section of the telencephalon with labeled ependymal cells (arrowhead) and cortices (arrows). (C) Details of the labeled ependymal cells (arrow). V: ventricle. (D) Cross-section of the diencephalon with messenger in the ependymal cells (arrow). (E) Cross-section of the mesencephalon. Labeling is on the cortex (arrows), commissural ansulata (ca), and basal nuclei (arrowhead). (F) Retina; MT-mRNA is in the outer and inner (*) nuclear layers and ganglion cells layer (arrow). (G, H) Liver with labeled Kupffer cells (arrows) and monocytes (arrowheads); unlabeled parenchymal cells (*). (I) Cross-section of the gut with labeled villi (v). (J) Labeled lung parenchyma (*). Bars: (A) 5 mm; (B, E) 200 μ m; (D, F) 100 μ m; (I, J) 50 μ m; (C, G) 20 μ m; (H) 10 μ m.

matter of debate. For example, it has been demonstrated that brain MTs', whereas having a role in defence against neurodegenerative disorders are also involved in neuronal regeneration and even in cognitive function by helping spatial learning and memory activities (Levin et al., 2006; Chung et al., 2008; West et al., 2008).

The unique lizard MT isoform seems to be involved both in detoxification and development. Indeed, these two functions are often combined also in other organisms. In mammals, for example, the ubiquitous MT-I and II isoforms play a role in detoxification processes and development of organs, including brain (Penkowa et al., '99).

In summary, under natural conditions, the "closed system" represented by the teleolecitic egg protects the developing embryo

from unwanted excess of metals, making MT expression and accumulation in detoxifying organs unnecessary until hatching when food intake begins. Additionally, the presence of MT transcripts in brain during development may ensure the correct final architecture of this organ that shows little adult plasticity.

Further studies will clarify the effect on MT gene expression of an unwanted intake of environmental pollutants in lizard eggs during incubation.

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