RESEARCH REPORT

Dynamic visualization of transcription and RNA subcellular localization in zebrafish

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ABSTRACT

Live imaging of transcription and RNA dynamics has been successful in cultured cells and tissues of vertebrates but is challenging to accomplish in vivo. The zebrafish offers important advantages to study these processes – optical transparency during embryogenesis, genetic tractability and rapid development. Therefore, to study transcription and RNA dynamics in an intact vertebrate organism, we have adapted the MS2 RNA-labeling system to zebrafish. By using this binary system to coexpress a fluorescent MS2 bacteriophage coat protein (MCP) and an RNA of interest tagged with multiple copies of the RNA hairpin MS2-binding site (MBS), live-cell imaging of RNA dynamics at single RNA molecule resolution has been achieved in other organisms. Here, using a Gateway-compatible MS2 labeling system, we generated stable transgenic zebrafish lines expressing MCP, validated the MBS-MCP interaction and applied the system to investigate zygotic genome activation (ZGA) and RNA localization in primordial germ cells (PGCs) in zebrafish. Although cleavage stage cells are initially transcriptionally silent, we detect transcription of MS2-tagged transcripts driven by the βactin promoter at ∼3.5 h post-fertilization, consistent with the previously reported ZGA. Furthermore, we show that MS2-tagged nanos3 3′UTR transcripts localize to PGCs, where they are diffusely cytoplasmic and within larger cytoplasmic accumulations reminiscent of those displayed by endogenous nanos3. These tools provide a new avenue for live-cell imaging of RNA molecules in an intact vertebrate. Together with new techniques for targeted genome editing, this system will be a valuable tool to tag and study the dynamics of endogenous RNAs during zebrafish developmental processes.

KEY WORDS: In vivo RNA labeling, Transcription, MS2, Transgenic zebrafish

INTRODUCTION

Given the vital roles coding and noncoding RNAs play in cell biology, it is not surprising that regulatory mechanisms coordinate every step of RNA metabolism, from transcription to translational capacity and, ultimately, degradation (Garneau et al., 2007; Holt and Bullock, 2009). To fully understand RNA regulation as it occurs in vivo requires detection and visualization of RNA in the living organism. Many tools have been used to study RNA dynamics in cell culture (Armitage, 2011; Santangelo et al., 2012); however, they have yet to be widely extended to studies in living vertebrates. One tool, the MS2 RNA-labeling system, based on the high-affinity binding of the bacteriophage MS2 coat protein (MCP) to its RNA hairpin binding site (MBS), has been used in model organisms (Bertrand et al., 1998; Belaya and St Johnston, 2011; Lionnet et al., 2011). By labeling a gene of interest with multiple copies of MBS and coexpressing a fluorescent MCP, live-cell imaging of RNA dynamics with single RNA molecule resolution is possible (Hocine et al., 2013). Tagging the fluorescent MCP with a nuclear localization signal (NLS) primes the MCP for interactions with nascent RNAs in the nucleus and reduces cytoplasmic background fluorescence, allowing enhanced visualization of cytoplasmic transcripts. This method has been used to study Drosophila oogenesis (Belaya and St Johnston, 2011) and embryogenesis (van Gemert et al., 2009), trafficking in Xenopus oocytes (Gagnon et al., 2013) and recently in brain slices of mice (Park et al., 2014) but has not been applied to vertebrate development. We have developed and applied a Gateway-based MS2-MCP system (Hartley et al., 2000; Walhout et al., 2000; Kwan et al., 2007; Villefranc et al., 2007) for the easy generation of expression vectors and stable transgenic zebrafish lines expressing fluorescent MCPs (FP-MCP). We have validated its use in zebrafish and have used it to study the onset of zygotic βactin transcription and nanos3 localization in PGCs. Together with established methods of transgenesis and mutagenesis, these tools should facilitate future studies of RNA regulation in living vertebrates.

RESULTS AND DISCUSSION

Transgenic NLS-4dMCP-GFP lines

We developed a set of Gateway-compatible plasmids to facilitate generation of MCP expression vectors (Fig. 1A). Using these plasmids and Tol2-mediated transgenesis (Kawakami et al., 1998, 2004; Kawakami, 2007), we generated stable transgenic zebrafish lines expressing MCP as a tandem dimer (tdMCP) (Wu et al., 2012) fused to a NLS and eGFP under the control of the ubiquitous βactin (Higashijima et al., 1997) and inducible hsp70 (Halloran et al., 2000) promoters (supplementary material Fig. S1). In all lines, the labeled cells displayed the expected nuclear fluorescence with minimal cytoplasmic background (supplementary material Fig. S1).

Validation of MCP-MBS interaction in zebrafish

To validate and test the feasibility of this system to visualize RNA molecules in zebrafish, we transiently and mosaically expressed cherry RNA tagged with MS2 hairpins using the βactin promoter (βactin:cherry-24xMBS) by injecting DNA into Tg(βactin:NLS-tdMCP-GFP) lines (Fig. 1B). Live imaging of zebrafish embryos revealed tdMCP-GFP cytoplasmic puncta in cells expressing the Cherry protein (Fig. 1C-E), likely representing cherry-24xMBS RNA species, as previously reported in other systems (Bertrand et al., 1998; van Gemert et al., 2009; Lionnet et al., 2011;
Schonberger et al., 2012; Gagnon et al., 2013; Park et al., 2014). Furthermore, time-lapse analysis of these cells revealed highly dynamic cytoplasmic puncta (supplementary material Movies 1 and 2) that were not detected in neighboring cells lacking the Cherry reporter (n=43 cells; 6 embryos). These experiments suggested that MS2-RNA labeling is feasible in zebrafish.

**Visualization of zygotic genome activation**

An advantage of MS2 labeling is the ability to track transcripts throughout their lifetime, because nuclear puncta appear soon after transcriptional activation (Larson et al., 2011; Lionnet et al., 2011; Park et al., 2014). We utilized this property to investigate the time course of zygotic genome activation (ZGA) in zebrafish. The zebrafish genome is quiescent until ~3 h post-fertilization (hpf) (Giraldez et al., 2006; Schier, 2007; Dalle Nogare et al., 2009; Tadros and Lipshitz, 2009), around the time of the mid-blastula transition (MBT). Development before this period is controlled by maternal factors, many of which are replaced by zygotic products after ZGA (Giraldez et al., 2006; Schier, 2007; Dalle Nogare et al., 2009; Park et al., 2014), the dotted line is independent of the MS2-MCP interaction. The dotted line not overlap with MCP-GFP cytoplasmic puncta, suggesting that this is independent of the MS2-MCP interaction. The dotted line denotes borders of cells expressing Cherry reporter. Scale bar: 10 µm.

**Fig. 1. Transgenic zebrafish lines expressing NLS-tdMCP-eGFP can be used to detect transcripts in vivo.** (A) Gateway compatible vectors for generation of NLS-tdMCP-FP and MS2-tagged RNAs. Plasmids used to generate transgenic lines by Tol2-mediated transgenesis are shown. 5′ Entry plasmids (p5E) containing the desired promoter elements can be recombined with NLS-tdMCP-eGFP or NLS-tdMCP-tagRFP entry plasmids (pME) for expression in any cell type or tissue. Similarly, the pME-NLS-tdMCP plasmid can be recombined with any in-frame fluorescent protein (FP) in a 3′ entry plasmid (p3E) to make custom NLS-tdMCP-FPs. RNA localization elements (LE) can be tagged with cherry-24xMBS by recombining the appropriate pME and p3E plasmids. (B) Schematic of the experiment used to validate in vivo MS2-MCP interactions. (C-E) Live imaging of embryos at the sphere stage shows that cytoplasmic puncta are visible only in cells expressing the Cherry reporter. In some cases, as shown in C, the Cherry reporter aggregates, but does not overlap with MCP-GFP cytoplasmic puncta, suggesting that the puncta represent transcriptional events. Where nuclear puncta were detected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus. Because DNA was injected, the number ranged from one to more than ten per nucleus.
MCP transgenic lines, the protein that detects transcripts (MCP) is maternally provided and present within the nucleus, and is thus poised to detect nascent transcripts. This design effectively eliminates lag between transcription, translation, and protein detection, as well as concerns that fluorescent protein stability might extend beyond the transcriptionally active period, common problems with conventional fluorescent protein transcriptional reporters. As evidence of this, Cherry reporter protein expression was not detectable at the blastula stages assayed when nuclear MBS–MCP-GFP puncta were first apparent, underscoring the usefulness of the system. Transcriptional nuclear puncta were detected in all cell types assayed (supplementary material Fig. S4). Of note, in Tg(βactin:NLs-tdMCP-GFP) transgenic embryos at gastrula stages and beyond, large globular nuclear accumulations became prominent, predominantly in skin cells (supplementary material Fig. S3A,B) and enveloping layer cells (Fig. 3B). These structures were qualitatively different and easily distinguishable from the transcriptional puncta observed in cells expressing MBS-tagged RNAs (supplementary material Fig. S3A,B). Combined with advances in targeted mutagenesis and targeted insertions into the zebrafish genome (Chang et al., 2013; Hruscha et al., 2013; Hwang et al., 2013a,b; Auer et al., 2014; Shin et al., 2014), it should be possible to insert MBS tags into endogenous loci to probe endogenous transcription in the future.

**Visualization of germ granule-like nanos3 RNA accumulations**

To determine whether the MS2 system could recapitulate the localization of an endogenous zebrafish RNA, we fused MS2 tags to the nanos3 3’UTR (Koprunner et al., 2001). nanos3 is required to maintain PGCs and localizes to them beginning after ZGA (Koprunner et al., 2001; Draper et al., 2007). This localization is achieved through 3’UTR-mediated stabilization of nanos3 transcripts in PGCs and microRNA (miRNA)-mediated degradation in somatic cells (Koprunner et al., 2001; Giraldez et al., 2006; Mishima et al., 2006). We injected cherry-24xMBS-nanos3 3’UTR RNA (hereafter called cherryMBSnos3’utr) into Tg(βactin:NLs-tdMCP-GFP) embryos and analyzed fixed embryos at time points before and after clearance of nanos3 RNA from somatic cells (Fig. 3A). Before complete clearance of the RNA, MCP-GFP was cytoplasmic in cells expressing the Cherry label (Fig. 3D,G), although no cytoplasmic signals were detected in uninjected embryos (Fig. 3B,E). Of note, MCP-GFP foci were also detected at the membrane between adjacent cells (Fig. 3D,G). Similar accumulations were detected in embryos injected with cherry-24xMBS RNA lacking a UTR (Fig. 3C,F), and in uninjected Tg(βactin:NLs-tdMCP-GFP) embryos, although they were less prominent (supplementary material Fig. S2). Thus, these accumulations represent a background artifact of expressing the NLStdMCP-GFP that becomes more prominent upon MBS-tagged RNA expression.

Similar to other fluorescent proteins tagged with the nanos3 3’UTR, cherryMBSnos3’utr, as indicated by Cherry expression by PGCs alone, was later specifically stabilized in the PGCs (Fig. 3I), reminiscent of endogenous nanos3, indicating that the MBS hairpins did not disrupt 3’UTR-mediated clearance and stabilization of the RNA (Mishima et al., 2006). In Cherry-positive PGCs, diffuse punctate cytoplasmic MCP-GFP revealed persisting RNA (n=99/122 Cherry-positive PGCs, 21 embryos), which was not visible in somatic cells (Fig. 3H-K). Diffuse cytoplasmic MCP-GFP was never observed in PGCs of uninjected embryos (n=0/101 PGCs, 15 embryos), although one or two small MCP-GFP accumulations were infrequently visible in perinuclear regions (n=19/101 PGCs, 15 embryos), which probably represent nuclear breakdown associated with mitosis, based on nuclear morphology. Similar punctate signals have been observed for nuclear membrane proteins in dividing PGCs in other studies (Strasser et al., 2008).

To test whether cherryMBSnos3’utr localization revealed by the cytoplasmic MCP-GFP resembled that of endogenous nanos3, we used RNAscope technology (Gross-Thebing et al., 2014) to probe nanos3 RNA. Similar to MS2-tagged RNA, nanos3 was diffusely cytoplasmic in PGCs but not in somatic cells (Fig. 4A,B). We also occasionally observed larger perinuclear accumulations of nanos3 (Fig. 4B). Closer examination of Cherry-positive PGCs from cherryMBSnos3’utr-injected embryos revealed that, in addition to diffuse cytoplasmic MCP-GFP, a fraction of Cherry-positive PGCs displayed perinuclear accumulations of MCP-GFP (Fig. 4C-F). To test whether these accumulations colocalized with endogenous nanos3, we again utilized RNAscope technology.
(Gross-Thebing et al., 2014) to examine nanos3 RNA. Importantly, to avoid detecting the injected cherryMBSnos3′utr, this probe specifically recognizes the open-reading frame of nanos3 and not the 3′UTR (Gross-Thebing et al., 2014). In injected embryos, endogenous nanos3 was again cytoplasmic in PGCs with occasional perinuclear accumulations (Fig. 4I). Based on MCP-GFP colocalization with endogenous nanos3 (Fig. 4G-J), we conclude that cherryMBSnos3′utr recapitulates endogenous nanos3 localization in PGCs and that the MCP-GFP fluorescence signal can serve to read out this RNA.

The nanos3 accumulations resembled the localization patterns of other germ cell markers that promote germ cell survival or differentiation and localize to perinuclear germ granules, such as Vasa (Knaut et al., 2000; Hartung et al., 2014) and Dead end (Weidinger et al., 2003; Slanchev et al., 2009). To test whether MCP-GFP colocalized with germ granules, cherryMBSnos3′utr-injected transgenic animals were immunostained for Vasa protein. In Cherry-positive PGCs some cytoplasmic accumulations of MCP-GFP coincided with Vasa-granules and others did not (Fig. 4K-N), which might have functional significance given the role of nanos3 in PGC maintenance (Draper et al., 2007). Moreover, this result suggests that distinct populations of germ granules exist and is consistent with previous findings that germ plasm RNAs differentially accumulate during blastula cleavage stages (Theusch et al., 2006).

Our studies illustrate the feasibility of using MS2 RNA labeling to study transcription and RNA localization in vivo in zebrafish. Given that a growing number of biologically important processes, including the regulation of developmental transitions such as ZGA and MBT, involve regulation by non-coding RNA species such as miRNAs and long non-coding RNAs (lncRNAs) (Giraldez et al., 2006; Pauli et al., 2011, 2012), this system provides the potential to detect such molecules in vivo. Furthermore, with a growing number of targeted genetic manipulation techniques and an increasing wealth of transgenic disease models, these tools should permit future studies of RNA dynamics in development and disease.

MATERIALS AND METHODS

Animals
AB strain wild-type zebrafish embryos were obtained from natural matings and reared according to standard procedures (Westerfield, 2000). Embryos and larvae were raised in 1× Embryo Medium at 28.5°C and were staged

Fig. 3. MS2-tagged nanos3 3′UTR is detected by MCP-GFP in the cytoplasm of PGCs. (A) Schematic depicting injection and imaging of cherry-24xMBS-nanos3 3′UTR RNA. cherry-24xMBS-nanos3 3′UTR is initially in all cells but later is maintained only in PGCs. (B-G) Imaging of fixed embryos reveals strictly nuclear MCP-GFP in uninjected embryos at the shield stage (B,E) and cytoplasmic RNA visualized by MCP-GFP puncta in cherry-24xMBS- and cherry-24xMBS-nos3 3′UTR-injected embryos (C,D,F,G). Punctate accumulations of MCP-GFP are present on cell membranes of (F) cherry-24xMBS- and (G) cherry-24xMBS-nos3 3′UTR-injected embryos (arrows) indicating this represents a background artifact. Scale bars: 20 µm. (H-K) The Cherry reporter reveals PGCs at 30 hpf in embryos that were injected with cherry-24xMBS-nos3′UTR. PGCs expressing Cherry display cytoplasmic MCP-GFP, whereas somatic cells and non-expressing PGCs have strictly nuclear MCP-GFP. The dotted lines denote borders of cells expressing Cherry reporter. Scale bar: 10 µm.
as described previously (Kimmel et al., 1995). All procedures and experimental protocols were in accordance with NIH guidelines and approved by the Einstein Institutional Animal Care and Use Committee (protocol number 20140502).

**Plasmids**

All primers are listed in supplementary material Table S1.

**MCP expression plasmids**

NLS-HA-tdMCP-GFP and NLS-HA-tdMCP-tagRFP were amplified by PCR from plasmids (Addgene, 40649) (Wu et al., 2012) and plasmids (Wu et al., 2012) using NLS-HA-tdMCP-FP-F+R primers. NLS-tdMCP was amplified by PCR from plasmids using NLS-tdMCPF+R primers. To add flanking attL1 and attL2 sites (Hartley et al., 2000; Walhout et al., 2000), PCR fragments were TOPO cloned into pCR8/GW/TOPO. To add flanking primers, NLS-tdMCP was amplified by PCR using NLS-HA-tdMCP-FP-F+R primers. NLS-HA-tdMCP-FP-F+R primers were TOPO cloned into pCR8/GW/TOPO.

**MBS-RNA expression plasmids**

pME-24xMBS was created from pCR4-24xMS2-SL (Addgene, 31865), which was digested with EcoRI, to release 24xMBS, which was then ligated into pCR8/GW/TOPO. pME-mCherry-24xMBS was created by amplifying mCherry with flanking BamHI primers, then ligating into pME-24xMBS. pCS-mCherry-24xMBS was created by recombining pME-mCherry-24xMBS with pCSDest (Addgene, 22423) (Villefranc et al., 2007). p3E-nanos3 3'UTR was created by amplifying the nanos3 3'UTR with flanking attB2R and attB3 sites by PCR from pSP64GFP3'UTRnos (Koprunner et al., 2001) using pSP64GFP3'UTRnos-attB2R+3 primers and then recombining with pDONR P2R-P3 (Invitrogen). pCS-mCherry-24xMBS-nanos3 3'UTR was created by recombining pME-mCherry-24xMBS and p3E-nanos3 3'UTR with pCSDest2 (Addgene, 22424) (Villefranc et al., 2007). To create pTol-factin:mCherry-24xMBS, p5E-factin and pME-mCherry-24xMBS were recombined with pTolDestR4-R2pA (Villefranc et al., 2007), and to create pTol-factin:mCherry, p5E-factin, and pME-mCherry, were recombined with pTolDestR4-R2pA.

**MCP stable transgenic lines**

To2 Transposase RNA, transcribed from pCS2FA-transposase (Kwan et al., 2007), and pTol-Promoter-NLS-tdMCP-eGFP circular DNA were combined (25 ng/μl each). Embryos were injected with 1 nl of this solution at the one-cell stage. GFP-expressing embryos were selected at 5 dpf and raised to generate founders (supplementary material Methods).

**Transient assays**

25 ng/μl each of circular pTol-factin-mCherry-24xMBS DNA and To2 Transposase RNA were combined and injected as described above. For live imaging, embryos were scored for mCherry expression and imaged. For the time-course analysis, embryos were fixed in 4% paraformaldehyde every half hour from 3 hpf to 4.5 hpf.

mCherry-24xMBS and mCherry-24xMBS-nanos3 3'UTR RNAs were transcribed from pCS-mCherry-24xMBS and pCS-mCherry-24xMBS-nanos3 3'UTR, respectively. Plasmids were linearized with NotI and transcribed with the mMESSAGE mMACHINE T7 transcription kit.
**References**


A transgenic mouse for in vivo detection of endogenous labeled mRNA. Nat. Methods 8, 165-170.


Movie 1. Cytoplasmic MCP-GFP signals are highly dynamic in blastula cells.

Transgenic embryos stably expressing NLS-tdMCP-GFP were injected with βactin:cherry-24xMBS DNA and live imaging was performed at a time following zygotic genome activation. In this ~5-6hpf embryo, nuclear puncta are observed representing active transcription. Highly dynamic cytoplasmic GFP signals are also observed, representing cytoplasmic cherry-24xMBS RNA molecules. The video represents a timelapse of a single z-plane. Scale bar, 10µm.
Movie 2. Cytoplasmic MCP-GFP signals are highly dynamic in skin cells.

Transgenic embryos stably expressing NLS-tdMCP-GFP were injected with βactin:cherry-24xMBS DNA and live imaging was performed at 24hpf. In this skin cell expressing the Cherry reporter (not shown) highly dynamic cytoplasmic GFP signals are observed, representing cytoplasmic cherry-24xMBS RNA molecules. The video represents a timelapse of a single z-plane. Scale bar, 10µm.
Movie 3. Nuclear MCP-GFP signals are dynamic.

Transgenic embryos stably expressing NLS-tdMCP-GFP were injected with βactin:cherry-24xMBS DNA and live imaging was performed at a time following zygotic genome activation. In this ~4-5hpf embryo, nuclear puncta are observed representing active transcription. Nuclear puncta can be seen to both disappear (top cell) and appear (bottom cell) over time. The bottom cell has just undergone a cell division and begun transcription. The video represents a timelapse of a z-projection. Scale bar, 10µm.
Movie 4. Nuclear MCP-GFP signals visualized exiting the nucleus.

Transgenic embryos stably expressing NLS-tdMCP-GFP were injected with βactin:cherry-24xMBS DNA and live imaging was performed at a time following zygotic genome activation. In this ~5-6hpf embryo, nuclear puncta are observed representing active transcription. GFP signals in the nucleus can be seen exiting the nucleus, presumably representing cherry-24xMBS RNA export. Three separate signals (blue, then yellow, then red arrowheads) appear in the nucleus and then seem to follow a similar route out of the nucleus and in the cytoplasm. The video represents a timelapse of a single z-plane. Scale bar, 5µm.
**Figure S1. Transgenic zebrafish lines expressing NLS-tdMCP-GFP**

(A) Animal pole view of live *Tg(βactin:NLS-tdMCP-GFP)* at 50% epiboly. Inset shows magnified view of white box illustrating nuclear expression of MCP-GFP. Stable β-actin lines displayed strong maternal and zygotic expression of NLS-tdMCP-GFP, with the ubiquitous GFP fluorescence gradually weakening through 3 days post-fertilization. Scale bars 50µm for main, 10µm for inset. n, nucleus. (B) Lateral view of live *Tg(hsp70:NLS-tdMCP-GFP)* at 48 hours post-fertilization (hpf) following 1 hour of 37°C heat shock at 24hpf shows ubiquitous expression of MCP-GFP. Stable hsp70 lines displayed strong ubiquitous expression following 1 hour of 37°C heat shock at 24hpf. Scale bar 50µm. MHB, midbrain-hindbrain boundary; A, anterior; P, posterior; D, dorsal; V, ventral.
Figure S2. No nuclear puncta are seen in uninjected embryos, though MCP-GFP puncta appear at cell membranes

Animal pole views of fixed Tg(βactin:NLS-tdMCP-GFP) uninjected embryos at (A) 3hpf and (B,C) 3.5hpf. Nuclear puncta are not apparent at any time point. However uninjected embryos also display accumulations of MCP-GFP at cell membranes (arrowheads). Scale bars (A,B) 50um and (C) 20um.
Figure S3. Appearance of nuclear puncta depends on the promoter sequence

Animal pole view of fixed Tg(βactin:NLS-tdMCP-GFP) embryos shows that embryos injected with DNA encoding MBS-tagged RNA driven by the βactin promoter results in nuclear puncta following ZGA at 4.5hpf (B) while DNA encoding MBS-tagged RNA driven by the ziwi promoter (Leu and Draper, 2010), a promoter element that is not activated at ZGA but instead drives transcription in the zebrafish germline beginning only at 7 days post-fertilization, does not (A). Embryos examined with nuclear puncta n=0/15. Scale bars 25µm.
Figure S4. Transcriptional puncta are seen in multiple cell types

After blastula stages, clusters of MCP (arrowheads) are particularly prevalent in enveloping layer and skin cells of un.injected Tg(βactin:NLS-tdMCP-GFP) embryos (A). However, transcriptional puncta (arrows) are readily distinguishable from the clusters in βactin:cherry-24xMBS DNA injected embryos due to their size and intensity (B). Transcriptional puncta are also evident in other cell types when βactin:cherry-24xMBS DNA is injected, including muscle cells, shown here (C,D). Scale bars, 5µm.
Table S1. Primers.

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<tr>
<th>Primer Name</th>
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<tr>
<td>NLS-HA-tdMCP-FP-R</td>
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Supplementary Materials and Methods

MCP stable transgenic lines

\(\beta\)-actin:NLS-tdMCP-eGFP transgenic fish: We identified 4 founders (33%) all of which produced embryos with ubiquitous expression, though varying levels, of NLS-tdMCP-eGFP. Line 2 showed the strongest expression, was propagated, and used for the studies herein. We propagated line 2 for over four generations and thus far have not noticed any change in expression levels or expression domains. None of the transgenic progeny of any of the founders displayed gross morphological defects.

hsp70l:NLS-tdMCP-eGFP transgenic fish: We identified 2 founders (17%) both of which gave rise to embryos with ubiquitous expression, though varying levels, of NLS-tdMCP-eGFP following 1h heat shock at 37°C at 24hpf. We propagated line hsp70l:NLS-tdMCP-eGFP-1 because it showed the highest expression. We have not noticed any change in expression levels or expression domains over 2 generations. None of the transgenic embryos from either founder displayed any gross morphological defects.
ziwi plasmids

*pTol-ziwi:mcherry-nanos3* 3’UTR was made by recombining *p5E-ziwi* (Leu and Draper, 2010), *pME-mCherry-24xMBS*, *p3E-nanos3 3’UTR* and *pDestTol2R4-R3pA* (Villefranc et al., 2007).

Movies

Transcriptional dynamics movies were obtained by taking a z-stack every minute of animal pole blastomeres. Cytoplasmic RNA dynamics movies were obtained by taking images from a single z-slice every second.