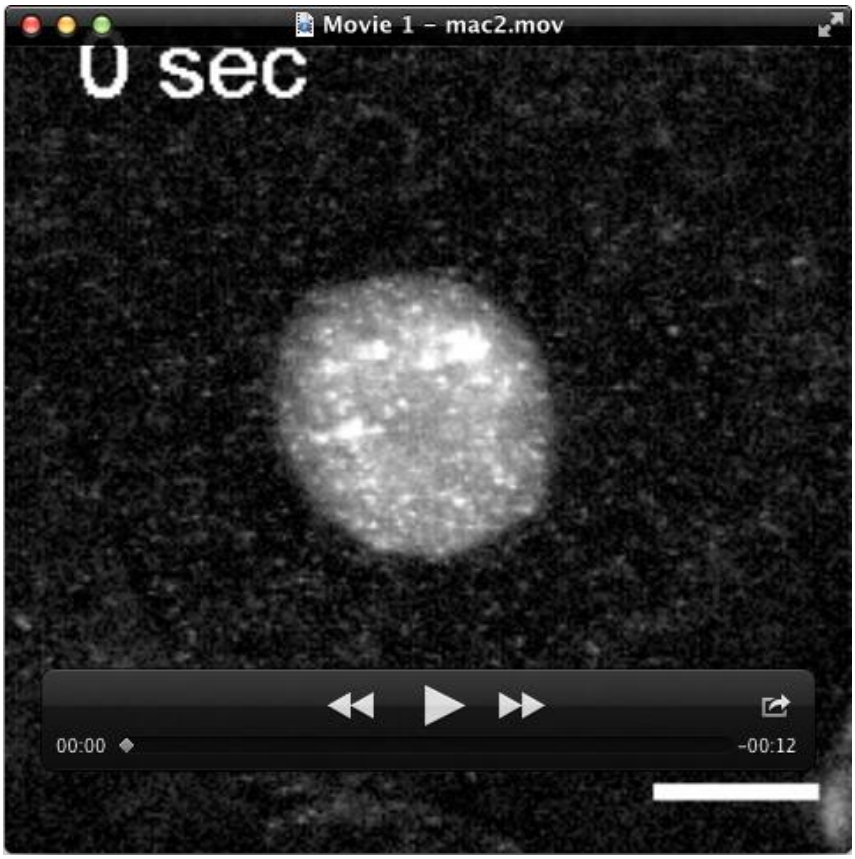
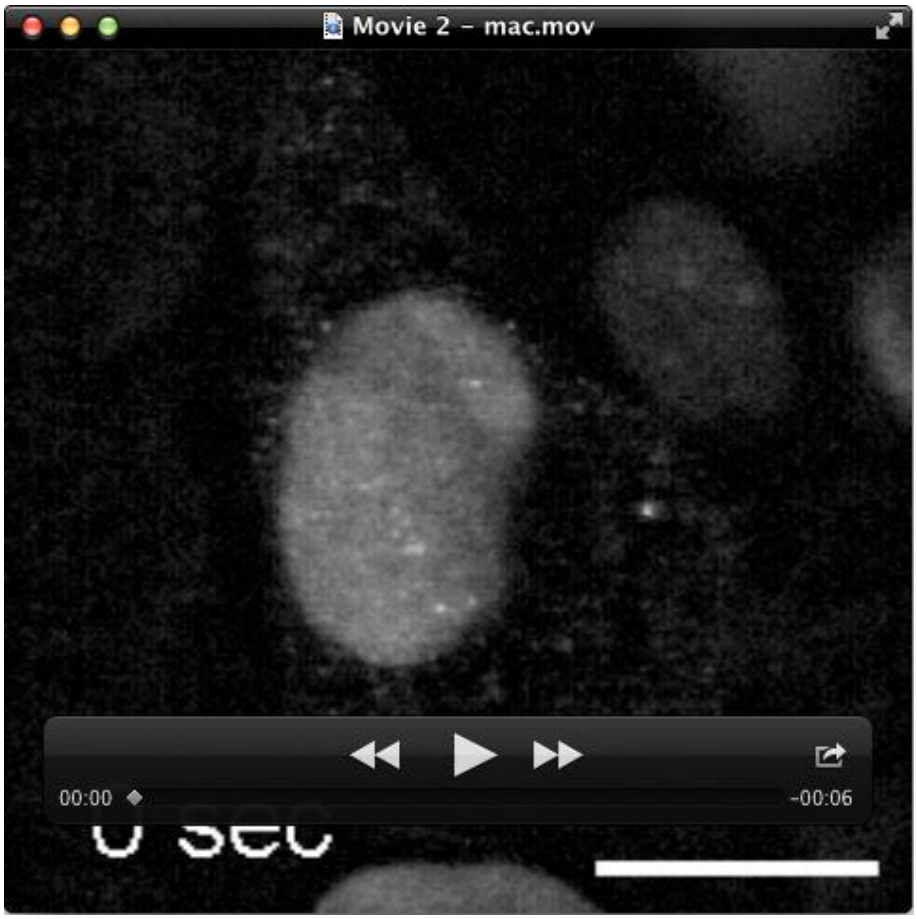


Movie 1. Cytoplasmic MCP-GFP signals are highly dynamic in blastula cells.

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.

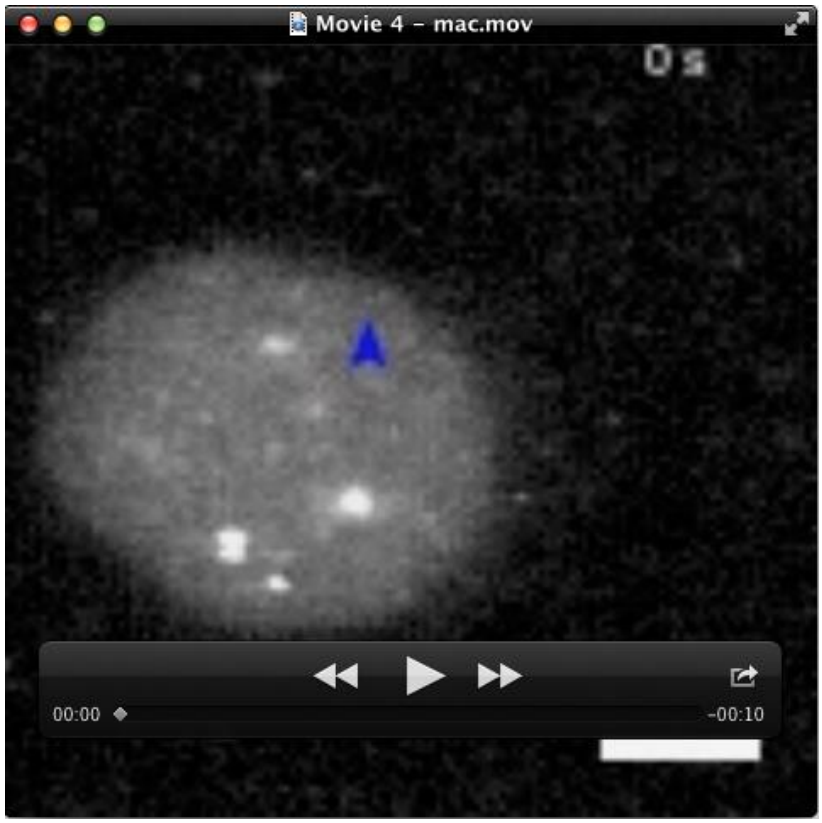


Movie 2. Cytoplasmic MCP-GFP signals are highly dynamic in skin cells.

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.

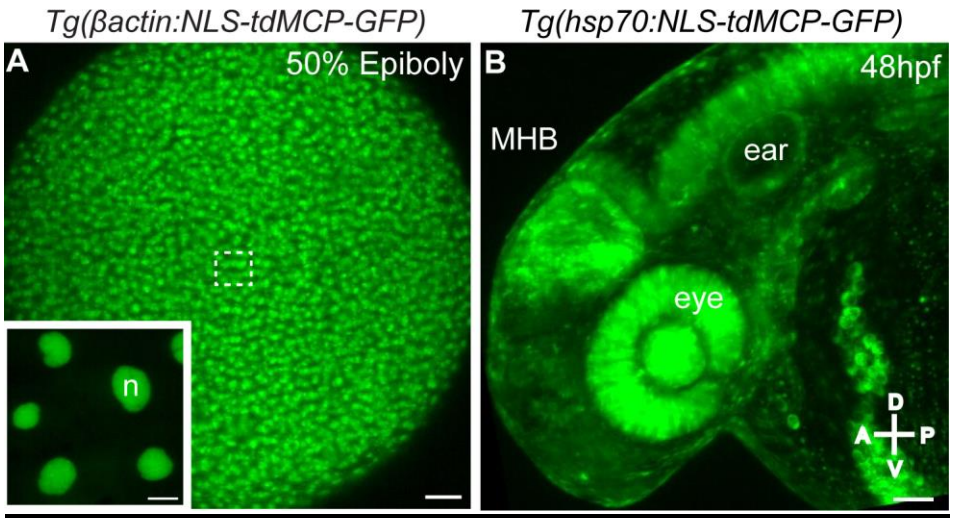


Figure S1. Transgenic zebrafish lines expressing NLS-tdMCP-GFP

(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 37ºC heat shock at 24hpf shows ubiquitous expression of MCP-GFP. Stable *hsp70* lines displayed strong ubiquitous expression following 1 hour of 37ºC heat shock at 24hpf. Scale bar 50µm. MHB, midbrain-hindbrain boundary; A, anterior; P, posterior; D, dorsal; V, ventral.

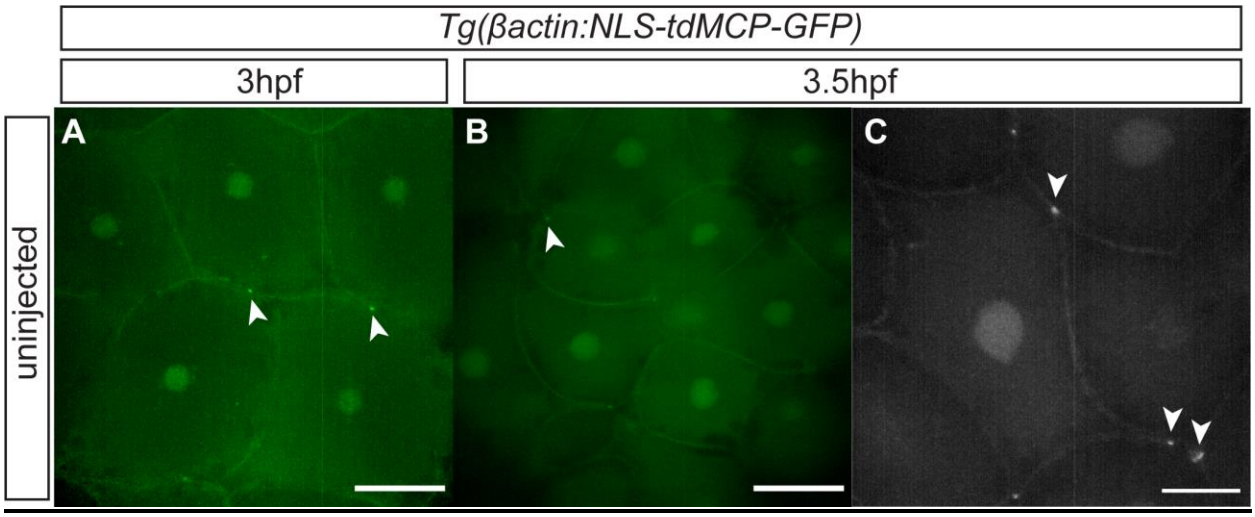


Figure S2. No nuclear puncta are seen in uninjected embryos, though MCP-GFP puncta appear at cell membranes

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.

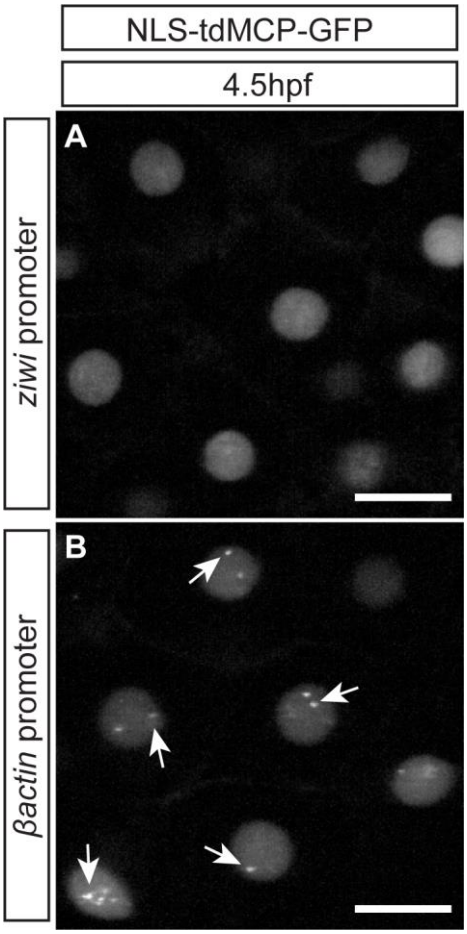


Figure S3. Appearance of nuclear puncta depends on the promoter sequence

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.

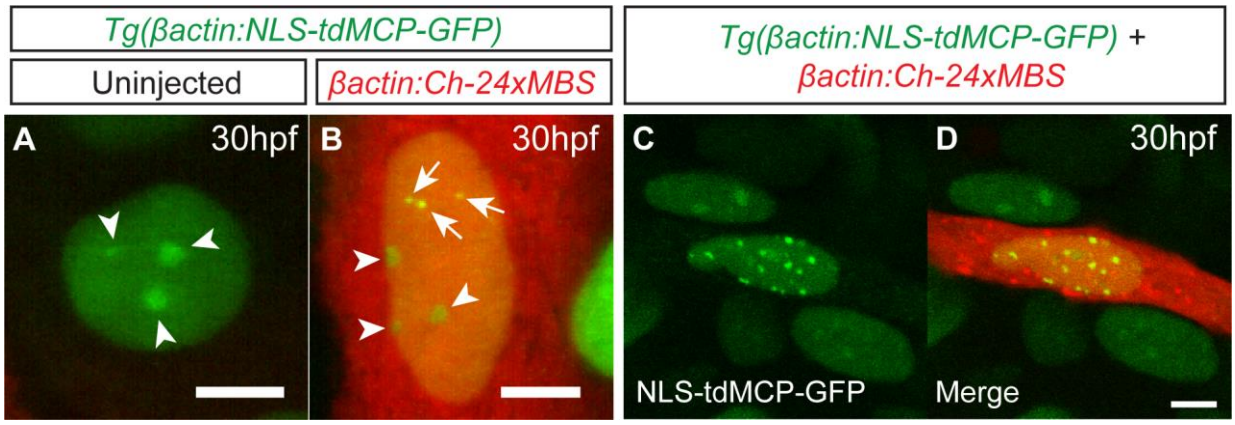


Figure S4. Transcriptional puncta are seen in multiple cell types

After blastula stages, clusters of MCP (arrowheads) are particularly prevalent in enveloping layer and skin cells of uninjected *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.

Primer Name	Sequence (5'-3')
NLS-HA-tdMCP-FP-F	GTCCCTTCTCGGCGATTCTG
NLS-HA-tdMCP-FP-R	TTACTTGTACAGCTCGTCCATGCC
NLS-tdMCP-F	GTCCCTTCTCGGCGATTCTG
NLS-tdMCP-R	CATTCTAGAATCCGCGTA
p3E-mCherry-BamHI-F	GATTCAGGATCCATGGTGAGCAAGGGCGAG
p3E-mCherry-BamHI-R	GATTCAGGATCCTTACTTGTACAGCTCGTC
pSP64GFP3'UTRnos-attB2R	GGGGACAGCTTTCTTGTACAAAGTGGAGCGGACA TTGATGCTCCG
pSP64GFP3'UTRnos-attB3	GGGGACAACTTTGTATAATAAAGTTGCACAGGAAA CAGCTATGACCATGA

Supplementary Materials and Methods

MCP stable transgenic lines

β-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 37ºC 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.