# Modelling of mRNA transport in nurse cells

Jonathan Harrison, Richard Parton, Ruth Baker

University of Oxford

## Abstract

Insert abstract information here

Insert Received for publication Date and in final form Date.

Insert Corresponding address and emails

## 1 Introduction

The localization of mRNA is crucial in a variety of biological contexts for the targeting of proteins to their site of function. This mechanism of gene expression is particularly relevant for polarized cells such as oocytes and early embryos. For example, the axes of *Drosophila Melongaster* are established through regulation of gradients in *bicoid* (*bcd*), *gurken* (*grk*), *oskar* (*osk*) and *nanos* (*nos*) mRNA (1). mRNA localization has been observed in a variety of species and cell types, including Drosophila and Xenopus oocytes, neurons, chicken fibroblasts, yeast and bacteria (2–6), thus demonstrating that this process is ubiquitous and not limited to large cells. Drosophila rely heavily on asymmetric locatization of mRNAs to coordinate the early development process both spatially and temporally.

Recent advances in imaging techniques and image analysis technologies (7–9) have allowed an advancement of our understanding of the mechanisms of mRNA localization. The use of flourescence in situ hybridisation (FISH) enables single molecules of mRNA to be labelled with high levels of sensitivity and specificity. The development of the MS2-MCP system, which consists of a MS2 bacteriophage RNA stem loop bound by MS2 coat protein fusion to a flourescent protein (10), has had great benefits for the imaging of live cells *in vivo*. The MS2-MCP system has been used successfully in the visualisation of *nos*, *grk*, *bcd* and *osk* mRNAs in Drosophila (11–14). Although there are limitations to these technologies dependent on cell type, they have permitted an improvement of our understanding of intracellular motility and structure. What has become clear is that a variety of different mechanisms are used to ensure localization of mRNAs.

### *1.1 Outline of mRNA localization process*

Once mRNA has been transcribed from DNA in the nucleus, the process by which it reaches its final site of localization, where it is then translated into protein, can be divided into four main stages: particle formation; nuclear export; transport; and anchoring. We will address each of these processes in turn.

mRNA does not exist on its own inside the cell. Instead the mRNA binds to proteins to form particle complexes, which are also known as ribonuclearproteins (RNPs). These proteins perform a range of different functions, including translational regulation to prevent translation while the mRNA is in transit, and may determine the final destination of the particle. Examples of proteins that form these paricles include *Squid* (*sqd*) and *Orb*. There is also evidence that RNPs are dynamically remodelled during the transport process (15).

After the RNA has formed into RNPs, it must diffuse through the crowded interchromatin spaces in the nucleus until it reaches a nuclear pore complex (NPC). The RNP is then exported out of the NPC into the cytoplasm via interactions with co-factors. Certain NPCs are more active than others at different times (15), but the overall direction of export out of the nucleus is unbiased (2).

The transport stage of mRNA localization occurs by different mechanisms for different mRNAs. The most common method observed is transport via molecular motors moving along the cytoskeleton (either microtubules or actin filaments). However, *nos* mRNA localizes using a diffusion and trapping technique (11) rather than by active transport. Active transport


0006-3495/08/09/2624/12 $2.00 doi:5

results in faster directed motion with velocities of the order of $1\mu ms^{-1}$ (13, 14), which is an order of magnitude quicker than movement by free diffusion. The motion of RNP complexes on microtubules is often non-uniform in nature, with bidirectionality observed by Vendra et al. (16) in Drosophila blastoderm embryos. It should be noted that, conventionally, individual types of molecular motors are thought to move unidirectionally, with Dynein directed towards the minus end of microtubules and Kinesin directed towards the plus end. The number of motors required for a given RNP complex can vary and may be governed by cis-acting localization elements in the mRNA (17). Possible explanations for the bidirectionality include: the bidirectional disorganized distribution of the microtubule network; a tug of war between different molecular motors moving in opposing directions on different microtubules; a tug of war between different motor species all bound to the same RNP complex; reversal of a molecular motor moving on a single microtubule, possibly due to regulation by microtubule associated proteins (MAPs) (18). The molecular motors must be correctly joined to their cargo and the motor cargo complex secured to the cytoskeleton. This is ensured by the linkers *Bicaudal D* (*BicD*) and *Egalitarian* (*Egl*) (10). Although the cytoskeleton was initially thought to be a stable static network, due how it was analysed in fixed material, it has recently been revealed to be a dynamic network with a biased random orientation of microtubules (19). This underlying structure had been suggested by the work of Zimyanin et al. (14) who observed RNPs containing *osk* mRNA moving in a biased random walk.

Once the RNP complex has reached its destination, it must be maintained in position by some anchoring mechanism to keep the mRNA localized within the cell. *grk* mRNA is anchored by the molecular motor Dynein (20) in the Drosophila oocyte and *nos* is trapped by actin (11). Dynein was observed by Delanoue and Davis (20) to act as a static motor without requiring further energy in the form of ATP to function. An alternative hypothesis is that continuous active transport is required to ensure the localization of mRNA, since Weil et al. (13) found that Dynein motor activity is required to ensure *bcd* localization in the anterior cortex of the Drosophila embryo.

## SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org.

## References


1. Wolpert, L., R. Beddington, J. Brockes, T. Jessell, P. Lawrence, and E. Meyerowitz, 1998. Principles of Development. Oxford University Press, Oxford.
2. Wilkie, G. S., and I. Davis, 2001. Drosophila wingless and pair-rule transcripts localize apically by dynein-mediated transport of RNA particles. Cell 105:209–219.
3. Bobola, N., R.-P. Jansen, T. H. Shin, and K. Nasmyth, 1996. Asymmetric accumulation of Ash1p in postanaphase nuclei depends on a myosin and restricts yeast mating-type switching to mother cells. Cell 84:699–709.
4. Mowry, K. L., and D. A. Melton, 1992. Vegetal messenger RNA localization directed by a 340-nt RNA sequence element in Xenopus oocytes. Science 255:991–994.
5. Rosbash, M., and R. H. Singer, 1993. RNA travel: tracks from DNA to cytoplasm. Cell 75:399–401.
6. Nevo-Dinur, K., A. Nussbaum-Shochat, S. Ben-Yehuda, and O. Amster-Choder, 2011. Translation-independent localization of mRNA in E. coli. Science 331:1081–1084.
7. Jeffery, W. R., C. R. Tomlinson, and R. D. Brodeur, 1983. Localization of actin messenger RNA during early ascidian development. Developmental biology 99:408–417.
8. Bertrand, E., P. Chartrand, M. Schaefer, S. M. Shenoy, R. H. Singer, and R. M. Long, 1998. Localization of ASH1 mRNA particles in living yeast. Molecular cell 2:437–445.
9. Hamilton, R. S., R. M. Parton, R. A. Oliveira, G. Vendra, G. Ball, K. Nasmyth, and I. Davis, 2010. ParticleStats: open source software for the analysis of particle motility and cytoskeletal polarity. Nucleic acids research 38:W641–W646.
10. Parton, R. M., A. Davidson, I. Davis, and T. T. Weil, 2014. Subcellular mRNA localisation at a glance. Journal of Cell Science 127:2127–2133.
11. Forrest, K. M., and E. R. Gavis, 2003. Live imaging of endogenous RNA reveals a diffusion and entrapment mechanism for nanos mRNA localization in Drosophila. Current biology 13:1159–1168.
12. Jaramillo, A. M., T. T. Weil, J. Goodhouse, E. R. Gavis, and T. Schupbach, 2008. The dynamics of fluorescently labeled endogenous gurken mRNA in Drosophila. Journal of cell science 121:887–894.
13. Weil, T. T., K. M. Forrest, and E. R. Gavis, 2006. Localization of bicoid mRNA in late oocytes is maintained by continual active transport. Developmental cell 11:251–262.
14. Zimyanin, V. L., K. Belaya, J. Pecreaux, M. J. Gilchrist, A. Clark, I. Davis, and D. St Johnston, 2008. In vivo imaging of oskar mRNA transport reveals the mechanism of posterior localization. Cell 134:843–853.
15. Weil, T. T., R. M. Parton, B. Herpers, J. Soetaert, T. Veenendaal, D. Xanthakis, I. M. Dobbie, J. M. Halstead, R. Hayashi, C. Rabouille, et al., 2012. Drosophila patterning is established by differential association of mRNAs with P bodies. Nature cell biology 14:1305–1313.

16. Vendra, G., R. S. Hamilton, and I. Davis, 2007. Dynactin suppresses the retrograde movement of apically localized mRNA in Drosophila blastoderm embryos. RNA 13:1860–1867.
17. Amrute-Nayak, M., and S. L. Bullock, 2012. Single-molecule assays reveal that RNA localization signals regulate dynein-dynactin copy number on individual transcript cargoes. Nature cell biology 14:416–423.
18. Buxbaum, A. R., H. Gal, and S. R. H, 2015. In the right place at the right time: visualizing and understanding mRNA localization. Nature Reviews Molecular Cell Biology 16:95–109.
19. Parton, R. M., R. S. Hamilton, G. Ball, L. Yang, C. F. Cullen, W. Lu, H. Ohkura, and I. Davis, 2011. A PAR-1–dependent orientation gradient of dynamic microtubules directs posterior cargo transport in the Drosophila oocyte. The Journal of cell biology 194:121–135.
20. Delanoue, R., and I. Davis, 2005. Dynein anchors its mRNA cargo after apical transport in the Drosophila blastoderm embryo. Cell 122:97–106.

## List of Figures

## List of Tables