BioMol Concepts, Vol. 1 (2010), pp. 59–66 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/BMC.2010.005

Review

The Musashi family RNA-binding proteins in stem cells

Kenichi Horisawa[1], Takao Imai[2], Hideyuki Okano[2] and Hiroshi Yanagawa[1,*]

[1] Department of Biosciences and Informatics, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan
[2] Department of Physiology, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan

* Corresponding author
e-mail: hyana@bio.keio.ac.jp

Abstract

The Musashi family is an evolutionarily conserved group of RNA-binding proteins. In mammal, two members of the group, Msi1 and Msi2, have been identified to date. Msi1 is considered to play roles in maintaining the stem cell status (stemness) of neural stem/progenitor cells in adults and in the development of central nervous system through translational regulation of its target mRNAs, which encode regulators of signal transduction and the cell cycle. Recently, strong expression of Msi1 in various somatic stem/progenitor cells of adult tissues, such as eye, gut, stomach, breast, and hair follicle, has been reported. The protein is also expressed in various cancer cells, and ectopically emerging cells have been found in neural tissues of patients with diseases involving neural disorder, including epilepsy. Many novel target mRNAs and regulatory pathways of Msi1 have been reported in recent years. Here, we present a review of the functions and action mechanisms of Msi1 protein and discuss possible directions for further study.

Keywords: Musashi; post-transcriptional regulation; progenitor cell; RNA-binding protein; stem cell.

Introduction

Recent findings demonstrate that post-transcriptional events, e.g., splicing, export, stabilization, localization, and translation, play an important role of life system and are highly orchestrated by various RNA-binding proteins (1). The Musashi family is a conserved group of RNA-binding proteins, which regulates translation of target mRNAs specifically (2, 3). The Musashi protein was originally identified as a regulator of asymmetrical division of sensory organ precursor cells in *Drosophila* (4). Subsequent studies established that Musashi is an RNA-binding protein bearing two typical RNA recognition motifs, and that it specifically binds to a sequence in the 3′ untranslated region (UTR) of *tramtrack69* (*ttk69*) mRNA (5); this binding prevents translation of *ttk69* mRNA, causing asymmetric cell division (6, 7). Thereafter, its mammalian homologs, Musashi1 (Msi1) and Musashi2 (Msi2), were found in mice (8, 9). Many Musashi and Musashi-like proteins have since been discovered in various multicellular animals, and overall the primary structure and expression pattern are highly conserved among them (10–16). Strong expression of Msi1 protein was observed in the nervous systems of vertebrates (17). In the central nervous system (CNS) of mouse, Msi1 protein is expressed in undifferentiated neural stem/precursor cells (NS/PCs) at the embryonic and adult stages (8, 17, 18). Molecular biological studies revealed that in mouse Msi1 in these cells controls the Notch signaling pathway through translational reduction of the m-Numb protein, an inhibitor of the Notch pathway, and thereby maintains the stem/progenitor cell status (19). Interestingly, expression of Msi1 was also observed in many types of somatic stem cells in adult tissues, e.g., eye (20), intestine (21), stomach (22), mammary gland (23), and hair follicle (24). Furthermore, significant expression of the protein was observed in proliferative cell populations of tumor tissues (25–27). This suggests a relationship between Msi1 and tumorigenesis. Although the above studies have indicated that the control of the Notch signal by Msi1 is important for stem cell maintenance (19), details of the molecular mechanisms and signaling network of Msi1 remain unclear. Recently, some novel target mRNAs, regulatory pathways, and functions of Msi1 have been identified, which could throw some light on these questions. In this review, we present an overview of the Musashi proteins, particularly mammalian Msi1, and consider possible directions for further research.

Discovery of the Musashi protein and its translational regulatory function

In loss-of-function experiments in *Drosophila*, Nakamura et al. found that the *musashi* gene is essential for asymmetrical division of sensory organ precursor cells, which are precursors of the ectodermal system common to both neural and non-neural cell lineages (4). In wild-type insects, a sensory organ precursor cell divides into a non-neural precursor cell and a neural precursor cell, whereas in *musashi* mutants two non-neural precursor cells are produced instead. The symmetrically divided non-neural precursor cells differentiate to hair-forming cells, leading to a double-bristle phenotype, instead of the single-hair wild-type phenotype.

Further studies revealed that the Musashi gene product, which is an RNA-binding protein, introduces neural differentiation potential for one daughter cell of the sensory organ

precursor cell by selective translational repression of mRNA of a neural differentiation inhibitory factor (a transcription repressor possessing a BTB domain and zinc finger domains) called *ttk69* (7). The ttk69 protein acts downstream of Notch as a determinant of non-neural identity. To identify specific target RNA motifs of the Musashi protein, Okabe et al. employed the *in vitro* selection method (SELEX method) (7) to address a synthesized random sequence RNA library. They found that the Musashi protein binds to sequences containing two or three (GUU…UAG) or (GUU…UG) repeats (7). It became clear that the *ttk69* mRNA contains 15 of these motif sequences in the 3′UTR, and it was confirmed that the Musashi protein binds to the 3′UTR of *ttk69* mRNA and inhibits translation of a reporter gene linked to the 3′UTR *in vitro* (7).

Musashi proteins maintain neural stem cells

Drosophila Musashi protein is also expressed in the compound eye primordium (6), in CNS (4), and in NS/PCs of larval brain (4), which have many characteristics in common with mammalian NS/PCs (29). Thus, to elucidate the functions of the Musashi gene family in mammals, a homolog search and immunohistochemical studies were performed in mice.

Two homolog genes, *musashi1* (*msi1*) (8) and *musashi2* (*msi2*) (9), were discovered in the mouse genome. Analyses showed strong expression of Msi1 in NS/PCs of the periventricular area (8, 17, 18). Thus, Msi1 can be used as a marker of NS/PCs in CNS of a variety of vertebrates. Indeed, such cells were identified in the adult human brain by using this approach (30). Detailed immunohistochemical analyses revealed that Msi1 is strongly expressed in the ventricular zone of the neural tube in embryo and in neurogenic sites within the postnatal brain, including the subventricular zone (SVZ), olfactory bulb, and rostral migratory stream (18). Msi1 protein is expressed in NS cells and progenitor cells within these tissues and is rapidly downregulated in postmitotic neurons (8). Mouse Msi2 protein is also a member of the Musashi family, displaying more than 90% homology with Msi1 protein in the RNA-binding domain (9). Its expression pattern in the CNS is very similar to that of Msi1. However, Msi2 is also continuously expressed in a subset of neuronal lineage cells, such as parvalbumin-containing GABA neurons in the neocortex and neurons in several nuclei of the basal ganglia (9).

To find target RNAs of Msi1 in mammals, a SELEX analysis was performed, as had been done in *Drosophila*, and the results revealed that the mouse Msi1 protein binds specifically to RNAs that possess a (G/A)UnAGU [n=1–3] sequence (19). A survey for the motif in mRNAs expressed in the embryonic CNS highlighted the 3′UTR of *m-numb* mRNA (31) as a candidate for the target. Subsequent experiments revealed that *m-munb* mRNA is a specific binding target of Msi1 protein *in vitro* and *in vivo*, and its translation is repressed by Msi1 protein (19, 32). The m-numb protein binds to the intracellular domain of Notch protein and inhibits the Notch signaling pathway (33), which positively regulates neural stem cell self-renewal (34–36). Kobayashi et al. reported that oscillation of expression of the *Hes1* gene, a downstream target of Notch, controls the differentiation of embryonic stem cells to neural cells (37). Taken together, these results indicate that Msi1 protein maintains stem cell status by enhancing the Notch signal through translational repression of *m-numb*. Indeed, Msi1 protein induces transactivation of the *Hes1* gene (19, 38). Thus, although the target mRNAs of mammalian and *Drosophila* Musashi protein differ, both proteins contribute to maintaining stem/progenitor cell status by translational repression of target mRNA.

By contrast, the function of Msi2 protein is still unclear, though it is known that Msi1 and Msi2 have similar RNA-binding specificity (9). The results of an Msi1 and Msi2 double-knockout experiment implied that these proteins have mutually complementary functions (39).

Somatic stem cells and Msi1 protein function

Recently, mammalian Msi1 protein expression was identified not only in CNS but also in other tissues and organs (Table 1). Raji et al. showed that Msi1 is produced in mouse eyes from embryonic stages until adulthood, and is also expressed in several unexpected sites, including the corneal epithelium and endothelium, stromal keratocytes, progenitor cells of the limbus, equatorial lens stem cells, differentiated lens epithelial cells, and differentiated lens fibers (20). A later study indicated that Msi1 knockout results in degeneration of photoreceptors and loss of visual cycle protein RPE65 in the microvilli of retinal pigment epithelium cells, which express Msi1 protein in wild-type animals (40). This could imply that Msi1 has an essential function for vision. Msi1 is also a marker of stem/progenitor cells in murine intestinal tissue, being located in the intestinal crypt, the putative location of stem cells (21, 41, 42). Recently, Murayama et al. observed that constitutive expression of Mis1 in intestinal epithelial cells suppressed expression of Paneth cell-specific genes, even though there was no significant effect on cell proliferation or on the Notch and Wnt signaling pathways (44). Using a similar strategy, Msi1 protein was detected in the isthmus/neck region of stomach, which is also a putative location of stem cells (22, 45). Furthermore, as well as other stem/progenitor cells, putative human breast stem cells were discovered in the mammary gland using specific antibodies for Msi1 and $p21^{WAF1}$ (23). Wang et al. found that much more committed progenitor cells in the mammary gland also express Msi1 protein, although luminal and myoepithelial progenitor cells do not express it (47). Likewise, Sugiyama-Nakagiri et al. reported that Msi1 and Msi2 are expressed in the epidermis and hair follicles of mice from E14.5 until adulthood (24). They hypothesized that Musashi proteins could have a function in hair cycle progression. Overall, expression of the Msi1 protein appears to be an effective marker for stem/progenitor cells in various tissues and is considered to regulate the stem cell status of cells.

Table 1 Msi1 expression in mammal tissues excluding CNS.

Tissue	Region or cells	Age	Species	References
Eye	Corneal epithelium, corneal endothelium, stromal keratocytes, progenitor cells of the limbus, equatorial lens stem cells, differentiated lens epithelial cells, differentiated lens fibers, retinal pigment epithelium cells	E12.5 – Adult	Mouse	Raji et al. (20) Susaki et al. (40)
Gut	Small intestinal crypt, colon crypt, columnar cells, epithelial cells	Embryo stage – Adult	Human, mouse, chicken	Kayahara et al. (41) Nishimura et al. (42) Potten et al. (21) Asai et al. (15) Samuel et al. (43) Murayama et al. (44)
Stomach	Luminal compartment of the mucosa, isthmus/neck region, fetal pyloric gland	Embryo stage – Adult	Rat, mouse, chicken	Nagata et al. (45) Akasaka et al. (22) Asai et al. (15) Murata et al. (46)
Mammary gland	Epithelial cells	Adult	Human, mouse	Clarke et al. (23)
Hair follicle	Keratinocyte	E14.5 – Adult	Human, mouse	Sugiyama-Nakagiri et al. (24)

Msi1 and diseases

Several reports suggest that Msi1 is involved in various diseases. A disease of great relevance to Msi1 is cancer, because many carcinoma cells are of epithelial stem cell lineage (48), expressing Msi1 protein. Strong expression of the Msi1 protein has been observed in many tumors, such as glioma (25), hepatoma (26), colorectal adenoma (27, 49), teratoid/rhabdoid tumors in eye (50), non-small cell lung cancer (51), retinoblastoma (52), medulloblastoma (53, 54), ependymoma (53), endometrial carcinoma (55), neurocytoma (56), glioblastoma (57), cervical carcinoma (58), etc. Although the exact function of Msi1 in these cancer cells remains unclear, knockdown of Msi1 by using siRNA resulted in tumor growth arrest in colon adenocarcinoma xenografts transplanted in athymic nude mice, reduced cancer cell proliferation, and increased apoptosis (59). These results suggest an important potential role of Msi1 in tumorigenesis and proliferation of tumors. It is known that some tumors express Msi2 protein, together with Msi1 (52). This could indicate a complementary role of the two proteins in tumors.

Other reports indicate that Msi1 is relevant to neurodegenerative disorders, such as Alzheimer's disease. Ectopic expression of Msi1 was observed in the hippocampus of Alzheimer's disease patients (60), whereas a significant decrease of Msi1-expressing cells was observed in the SVZ of patients (61). Although it is difficult to explain these phenomena at present, the function of Msi1 in maintaining stemness of the NS/PCs might play a role in the pathogenesis of the disease.

Recently, Oki et al. report upregulation of Msi1 expression in collapsed nervous system tissue arising from a blood circulation defect. In ischemic striatum induced by middle cerebral artery occlusion (MCAO), an increase in Msi1-immunoreactivity was observed in reactive astrocytes from 2 days after MCAO, persisting until 14 days. The proliferation of Msi1-positive cells was observed from 4 days after MCAO and reached a peak at 7 days (62).

Interestingly, Msi1 protein-expressing cells are increased in the hippocampus of mesial temporal lobe epilepsy (MTLE) patients (63). Large numbers of Msi1-positive cells were also observed in the SVZ in these patients (63). Increased neurogenesis has been reported in animal models of MTLE (63). Abnormal proliferation of such Msi1-expressing neural progenitors in hippocampus might cause epilepsy.

Current insights into the functions of Msi1

Recently, the molecular mechanism of translational repression by the Msi1 protein has been uncovered. Kawahara et al. identified poly(A)-binding protein (PABP) as an Msi1-binding protein, and found that Msi1 competes with elF4G for PABP binding on its target mRNAs (Figure 1) (32). By contrast, Charlesworth et al. found a novel function of Musashi in *Xenopus* oocytes: it activates translation of mRNA of *mos* (64), which is a gene related to meiotic cell cycle progression (65). This is a conflicting result from previous findings on the translational effect of Msi1. However, in human oocytes, a parallel physiological phenomenon is controlled by other machinery instead of the Musashi homolog proteins (66). In other words, although Musashi family proteins are highly conserved among vertebrates, their precise roles might be species-dependent. Examination of the evolutionary conservation of the 3′UTR of target mRNAs will be an important point in analysis of the regulatory network of RNA-binding proteins.

Until recently, only a few direct target mRNAs of mammal Msi1 (19, 67) had been reported. de Sousa Abreu et al. performed an RNA immunoprecipitation (RIP)-Chip assay in HEK293T cells to identify the target mRNAs comprehensively (68). They identified a group of 64 mRNAs, whereby the genes belong to two main functional categories pertinent to tumorigenesis: (i) cell cycle, proliferation, differentiation, and apoptosis and (ii) protein modification. Interestingly, subsequent proteomics study revealed that Msi1 can have not only negative but also positive effects on gene expression for some of the targets (68). This is consistent with the results in *Xenopus* oocytes (64).

Our group also performed an *in vitro* screening analysis to detect specific binding targets of Msi1 controlling the stem cell status of NS cells. We succeeded in finding a novel target mRNA of Msi1, *doublecortin* (*dcx*), from an mRNA library of embryonic mouse brain tissue (69). *dcx* is a gene related to migration of newborn neurons and neural development, and mutation in this gene cause an X-linked dominant disorder characterized by classic lissencephaly with severe mental retardation and epilepsy in hemizygous males and subcortical laminar heterotopia, also known as double cortex syndrome, associated with milder mental retardation and epilepsy, in heterozygous females (70–72). The Msi1 protein specifically bound *in vitro* to the 3′UTR region of the mRNA, which contains an Msi1 binding motif, and repressed translation of a reporter gene linked to the mRNA fragment (69). We hypothesize that the Msi1 protein prevents inappropriate migration of NS cells through translational inhibition of the *dcx* gene. Several findings support our hypothesis: firstly, the Dcx protein is expressed only in neuronal precursors just differentiated from NS/PCs (73); secondly, mutually exclusive 'protein' expression of Msi1 and Dcx in human brain was observed (63); and thirdly, knockout of Musashi family genes reduced the number of neurospheres isolated from embryonic mouse brains, whereas knockdown of *dcx* prevented migration of cells from neurospheres, leaving their structure intact (74). In addition, we have also found another candidate Msi1-binding mRNA, which is related to neuronal migration and axon outgrowth (unpublished data). Thus, Msi1 might repress the maturation of NS/PCs to neurons through direct translational inhibition of genes that influence neuronal maturation and migration.

The precise mechanism through which the function of Msi1 is controlled remains unclear. Although Wang et al. proposed that the Msi1 protein is involved in both Notch and Wnt signaling pathways as a novel autocrine process (45, 75), details of the mechanism remain unclear and direct regulators of Msi1 have not been identified. By contrast, Ratti et al. reported post-transcriptional regulation of Msi1 mRNA by ELAV, an RNA-binding protein of *Drosophila* (76). This is an interesting result, because it could imply that some type of cascade of post-transcriptional regulation contributes to neurogenesis, in addition to other machinery, i.e., signal transduction, transcriptional regulation, and post-translational modification.

Expert opinion and outlook

The Musashi family is a highly conserved RNA-binding protein group. *Drosophila* Musashi protein and its mouse homolog, Msi1, work as translational repressors of specific target mRNAs including *ttk69*, *m-numb*, and $p21^{WAF1}$. The Msi1 protein is expressed in stem/progenitor cell lineages of various tissues and organs. The physiological function of the Msi1 is considered as a key to the maintenance of the stemness of stem/progenitor cells. Although recent studies have revealed that a Notch signal inhibitor, *m-numb*, and a cell cycle regulator, $p21^{WAF1}$, are direct targets of Msi1, and the machinery involved is located in the context of both the Notch and Wnt signaling pathways (75), the details remain

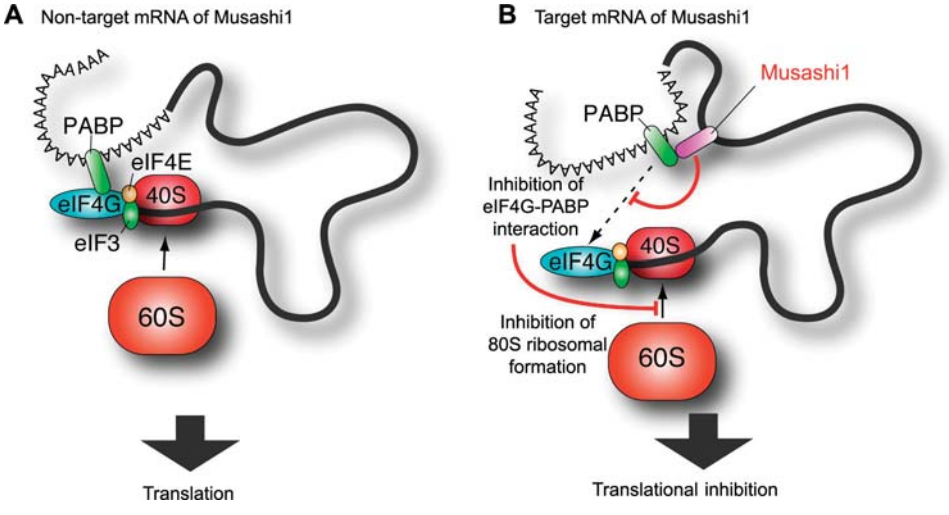

Figure 1 Schematic representation of the function of Musashi1.
Musashi1 interacts with the 3′UTR of its target mRNA and PABP, and subsequently inhibits translation initiation by competing with eIF4G for PABP. These sequential events inhibit formation of the 80S ribosome complex.

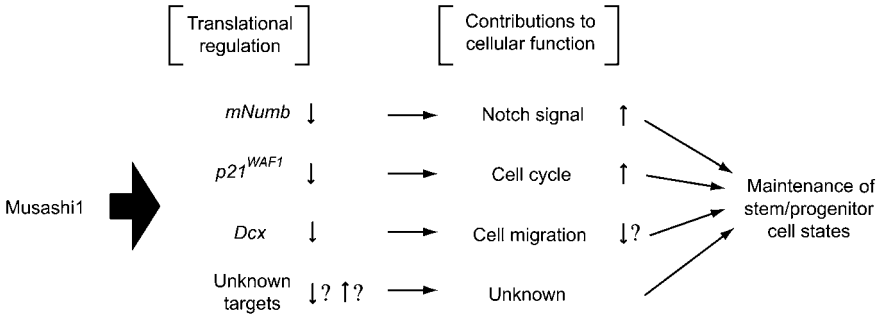

Figure 2 Multiple pathways to maintain stem/progenitor cell states by Musashi1.
Translational inhibition of various targets genes by Musashi1 might maintain the stem/progenitor cell states syntagmatically.

to be uncovered. RIP-Chip analysis identified many candidates for the target mRNAs of Msi1, and the translation of some of them was activated by Msi1 protein (68). These results appear to be in opposition to previous findings that Msi1 represses the translation of its targets (32) and imply the existence of another molecular machinery that activates translation of targets. Components of this machinery, e.g., binding proteins of Msi1, need to be clarified comprehensively by means of high-throughput techniques (77–79).

By contrast, we also found a candidate target mRNA of Msi1 (69), which is expressed specifically in young neurons just differentiated from NS/PCs (73). This suggests that screening studies using generic cultured cells, such as HEK293T, would be insufficient to find targets specifically expressed in stem/progenitor cells in tissues. Isolated somatic stem/progenitor cells or tissues which contain these types of cells should be used as biological sources for screening studies to find target mRNAs. We speculate that Msi1 might have specific targets in each cell type or site, such as *dcx*, in addition to the previously discovered targets, which are related to cell cycle, proliferation, and self-renewal (Figure 2). *In silico* screening for target mRNAs, based on the motif search and 2D structure prediction (80), could also be an alternative or complementary approach, as well as other high-throughput screening methods for protein-RNA interactions (68, 81, 82).

Finally, cooperation and division of roles between Msi1 and Msi2 proteins should be elucidated to fully understand the physiological functions of the Musashi family.

References

1. Keene JD. RNA regulons: coordination of post-transcriptional events. Nat Rev Genet 2007; 8: 533–43.
2. Okano H, Imai T, Okabe M. Musashi: a translational regulator of cell fate. J Cell Sci 2002; 115: 1355–9.
3. Okano H, Kawahara H, Toriya M, Nakao K, Shibata S, Imai T. Function of RNA-binding protein Musashi-1 in stem cells. Exp Cell Res 2005; 306: 349–56.
4. Nakamura M, Okano H, Blendy JA, Montell C. Musashi, a neural RNA-binding protein required for Drosophila adult external sensory organ development. Neuron 1994; 13: 67–81.
5. Lai ZC, Li Y. Tramtrack69 is positively and autonomously required for Drosophila photoreceptor development. Genetics 1999; 152: 299–305.
6. Hirota Y, Okabe M, Imai T, Kurusu M, Yamamoto A, Miyao S, Nakamura M, Sawamoto K, Okano H. Musashi and seven in absentia downregulate Tramtrack through distinct mechanisms in Drosophila eye development. Mech Dev 1999; 87: 93–101.
7. Okabe M, Imai T, Kurusu M, Hiromi Y, Okano H. Translational repression determines a neuronal potential in Drosophila asymmetric cell division. Nature 2001; 411: 94–8.
8. Sakakibara S, Imai T, Hamaguchi K, Okabe M, Aruga J, Nakajima K, Yasutomi D, Nagata T, Kurihara Y, Uesugi S, Miyata T, Ogawa M, Mikoshiba K, Okano H. Mouse-Musashi-1, a neural RNA-binding protein highly enriched in the mammalian CNS stem cell. Dev Biol 1996; 176: 230–42.
9. Sakakibara S, Nakamura Y, Satoh H, Okano H. RNA-binding protein Musashi2: developmentally regulated expression in neural precursor cells and subpopulations of neurons in mammalian CNS. J Neurosci 2001; 21: 8091–107.
10. Good P, Yoda A, Sakakibara S, Yamamoto A, Imai T, Sawa H, Ikeuchi T, Tsuji S, Satoh H, Okano H. The human Musashi homolog 1 (MSI1) gene encoding the homologue of Musashi/Nrp-1, a neural RNA-binding protein putatively expressed in CNS stem cells and neural progenitor cells. Genomics 1998; 52: 382–4.
11. Yoda A, Sawa H, Okano H. MSI-1, a neural RNA-binding protein, is involved in male mating behaviour in Caenorhabditis elegans. Genes Cells 2000; 5: 885–95.
12. Kawashima T, Murakami AR, Ogasawara M, Tanaka K, Isoda R, Sasakura Y, Nishikata T, Okano H, Makabe KW. Expression patterns of musashi homologs of the ascidians, Halocynthia roretzi and Ciona intestinalis. Dev Genes Evol 2000; 210: 162–5.
13. Cuadrado A, García-Fernández LF, Imai T, Okano H, Muñoz A. Regulation of tau RNA maturation by thyroid hormone is mediated by the neural RNA-binding protein musashi-1. Mol Cell Neurosci 2002; 20: 198–210.
14. Lowe CJ, Wu M, Salic A, Evans L, Lander E, Stange-Thomann N, Gruber CE, Gerhart J, Kirschner M. Anteroposterior patterning in hemichordates and the origins of the chordate nervous system. Cell 2003; 113: 853–65.
15. Asai R, Okano H, Yasugi S. Correlation between Musashi-1 and c-hairy-1 expression and cell proliferation activity in the developing intestine and stomach of both chicken and mouse. Dev Growth Differ 2005; 47: 501–10.
16. Higuchi S, Hayashi T, Tarui H, Nishimura O, Nishimura K, Shibata N, Sakamoto H, Agata K. Expression and functional

analysis of musashi-like genes in planarian CNS regeneration. Mech Dev 2008; 125: 631–45.
17. Kaneko Y, Sakakibara S, Imai T, Suzuki A, Nakamura Y, Sawamoto K, Ogawa Y, Toyama Y, Miyata T, Okano H. Musashi1: an evolutionally conserved marker for CNS progenitor cells including neural stem cells. Dev Neurosci 2000; 22: 139–53.
18. Sakakibara S, Okano H. Expression of neural RNA-binding proteins in the postnatal CNS: implications of their roles in neuronal and glial cell development. J Neurosci 1997; 17: 8300–12.
19. Imai T, Tokunaga A, Yoshida T, Hashimoto M, Mikoshiba K, Weinmaster G, Nakafuku M, Okano H. The neural RNA-binding protein Musashi1 translationally regulates mammalian numb gene expression by interacting with its mRNA. Mol Cell Biol 2001; 21: 3888–900.
20. Raji B, Dansault A, Leemput J, de la Houssaye G, Vieira V, Kobetz A, Arbogast L, Masson C, Menasche M, Abitbol M. The RNA-binding protein Musashi-1 is produced in the developing and adult mouse eye. Mol Vis 2007; 13: 1412–27.
21. Potten CS, Booth C, Tudor GL, Booth D, Brady G, Hurley P, Ashton G, Clarke R, Sakakibara S, Okano H. Identification of a putative intestinal stem cell and early lineage marker; musashi-1. Differentiation 2003; 71: 28–41.
22. Akasaka Y, Saikawa Y, Fujita K, Kubota T, Ishikawa Y, Fujimoto A, Ishii T, Okano H, Kitajima M. Expression of a candidate marker for progenitor cells, Musashi-1, in the proliferative regions of human antrum and its decreased expression in intestinal metaplasia. Histopathology 2005; 47: 348–56.
23. Clarke RB, Spence K, Anderson E, Howell A, Okano H, Potten CS. A putative human breast stem cell population is enriched for steroid receptor-positive cells. Dev Biol 2005; 277: 443–56.
24. Sugiyama-Nakagiri Y, Akiyama M, Shibata S, Okano H, Shimizu H. Expression of RNA-binding protein Musashi in hair follicle development and hair cycle progression. Am J Pathol 2006; 168: 80–92.
25. Toda M, Iizuka Y, Yu W, Imai T, Ikeda E, Yoshida K, Kawase T, Kawakami Y, Okano H, Uyemura K. Expression of the neural RNA-binding protein Musashi1 in human gliomas. Glia 2001; 34: 1–7.
26. Shu HJ, Saito T, Watanabe H, Ito JI, Takeda H, Okano H, Kawata S. Expression of the Musashi1 gene encoding the RNA-binding protein in human hepatoma cell lines. Biochem Biophys Res Commun 2002; 293: 150–4.
27. Sakatani T, Kaneda A, Iacobuzio-Donahue CA, Carter MG, de Boom Witzel S, Okano H, Ko MS, Ohlsson R, Longo DL, Feinberg AP. Loss of imprinting of Igf2 alters intestinal maturation and tumorigenesis in mice. Science 2005; 307: 1976–8.
28. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 1990; 249: 505–10.
29. Ito K, Hotta Y. Proliferation pattern of postembryonic neuroblasts in the brain of Drosophila melanogaster. Dev Biol 1992; 149: 134–48.
30. Pincus DW, Keyoung HM, Harrison-Restelli C, Goodman RR, Fraser RA, Edgar M, Sakakibara S, Okano H, Nedergaard M, Goldman SA. Fibroblast growth factor-2/brain-derived neurotrophic factor-associated maturation of new neurons generated from adult human subependymal cells. Ann Neurol 1998; 43: 576–85.
31. Zhong W, Feder JN, Jiang MM, Jan LY, Jan YN. Asymmetric localization of a mammalian numb homolog during mouse cortical neurogenesis. Neuron 1996; 17: 43–53.
32. Kawahara H, Imai T, Imataka H, Tsujimoto M, Matsumoto K, Okano H. Neural RNA-binding protein Musashi1 inhibits translation initiation by competing with eIF4G for PABP. J Cell Biol 2008; 181: 639–53.
33. Berdnik D, Török T, González-Gaitán M, Knoblich JA. The endocytic protein alpha-Adaptin is required for numb-mediated asymmetric cell division in Drosophila. Dev Cell 2002; 3: 221–31.
34. Nakamura Y, Sakakibara S, Miyata T, Ogawa M, Shimazaki T, Weiss S, Kageyama R, Okano H. The bHLH gene hes1 as a repressor of the neuronal commitment of CNS stem cells. J Neurosci 2000; 20: 283–93.
35. Hitoshi S, Alexson T, Tropepe V, Donoviel D, Elia AJ, Nye JS, Conlon RA, Mak TW, Bernstein A, van der Kooy D. Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. Genes Dev 2002; 16: 846–58.
36. Tokunaga A, Kohyama J, Yoshida T, Nakao K, Sawamoto K, Okano H. Mapping spatio-temporal activation of Notch signaling during neurogenesis and gliogenesis in the developing mouse brain. J Neurochem 2004; 90: 142–54.
37. Kobayashi T, Mizuno H, Imayoshi I, Furusawa C, Shirahige K, Kageyama R. The cyclic gene Hes1 contributes to diverse differentiation responses of embryonic stem cells. Genes Dev 2009; 23: 1870–5.
38. Yokota N, Mainprize TG, Taylor MD, Kohata T, Loreto M, Ueda S, Dura W, Grajkowska W, Kuo JS, Rutka JT. Identification of differentially expressed and developmentally regulated genes in medulloblastoma using suppression subtraction hybridization. Oncogene 2004; 23: 3444–53.
39. Sakakibara S, Nakamura Y, Yoshida T, Shibata S, Koike M, Takano H, Ueda S, Uchiyama Y, Noda T, Okano H. RNA-binding protein Musashi family: roles for CNS stem cells and a subpopulation of ependymal cells revealed by targeted disruption and antisense ablation. Proc Natl Acad Sci USA 2002; 99: 15194–9.
40. Susaki K, Kaneko J, Yamano Y, Nakamura K, Inami W, Yoshikawa T, Ozawa Y, Shibata S, Matsuzaki O, Okano H, Chiba C. Musashi-1, an RNA-binding protein, is indispensable for survival of photoreceptors. Exp Eye Res 2009; 88: 347–55.
41. Kayahara T, Sawada M, Takaishi S, Fukui H, Seno H, Fukuzawa H, Suzuki K, Hiai H, Kageyama R, Okano H, Chiba T. Candidate markers for stem and early progenitor cells, Musashi-1 and Hes1, are expressed in crypt base columnar cells of mouse small intestine. FEBS Lett 2003; 535: 131–5.
42. Nishimura S, Wakabayashi N, Toyoda K, Kashima K, Mitsufuji S. Expression of Musashi-1 in human normal colon crypt cells: a possible stem cell marker of human colon epithelium. Dig Dis Sci 2003; 48: 1523–9.
43. Samuel S, Walsh R, Webb J, Robins A, Potten C, Mahida YR. Characterization of putative stem cells in isolated human colonic crypt epithelial cells and their interactions with myofibroblasts. Am J Physiol Cell Physiol 2009; 296: C296–305.
44. Murayama M, Okamoto R, Tsuchiya K, Akiyama J, Nakamura T, Sakamoto N, Kanai T, Watanabe M. Musashi-1 suppresses expression of Paneth cell-specific genes in human intestinal epithelial cells. J Gastroenterol 2009; 44: 173–82.
45. Nagata H, Akiba Y, Suzuki H, Okano H, Hibi T. Expression of Musashi-1 in the rat stomach and changes during mucosal injury and restitution. FEBS Lett 2006; 580: 27–33.
46. Murata H, Tsuji S, Tsujii M, Nakamura T, Fu HY, Eguchi H, Asahi K, Okano H, Kawano S, Hayashi N. Helicobacter pylori infection induces candidate stem cell marker Musashi-1 in the human gastric epithelium. Dig Dis Sci 2008; 53: 363–9.
47. Wang XY, Yin Y, Yuan H, Sakamaki T, Okano H, Glazer RI. Musashi1 modulates mammary progenitor cell expansion

through proliferin-mediated activation of the Wnt and Notch pathways. Mol Cell Biol 2008; 28: 3589–99.
48. Miller SJ, Lavker RM, Sun TT. Interpreting epithelial cancer biology in the context of stem cells: tumor properties and therapeutic implications. Biochim Biophys Acta 2005; 1756: 25–52.
49. Schulenburg A, Cech P, Herbacek I, Marian B, Wrba F, Valent P, Ulrich-Pur H. CD44-positive colorectal adenoma cells express the potential stem cell markers musashi antigen (msi1) and ephrin B2 receptor (EphB2). J Pathol 2007; 213: 152–60.
50. Fujita M, Sato M, Nakamura M, Kudo K, Nagasaka T, Mizuno M, Amano E, Okamoto Y, Hotta Y, Hatano H, Nakahara N, Wakabayashi T, Yoshida J. Multicentric atypical teratoid/rhabdoid tumors occurring in the eye and fourth ventricle of an infant: case report. J Neurosurg 2005; 102: 299–302.
51. Kanai R, Eguchi K, Takahashi M, Goldman S, Okano H, Kawase T, Yazaki T. Enhanced therapeutic efficacy of oncolytic herpes vector G207 against human non-small cell lung cancer – expression of an RNA-binding protein, Musashi1, as a marker for the tailored gene therapy. J Gene Med 2006; 8: 1329–40.
52. Seigel GM, Hackam AS, Ganguly A, Mandell LM, Gonzalez-Fernandez F. Human embryonic and neuronal stem cell markers in retinoblastoma. Mol Vis 2007; 13: 823–32.
53. Nakano A, Kanemura Y, Mori K, Kodama E, Yamamoto A, Sakamoto H, Nakamura Y, Okano H, Yamasaki M, Arita N. Expression of the neural RNA-binding protein Musashi1 in pediatric brain tumors. Pediatr Neurosurg 2007; 43: 279–84.
54. Sanchez-Diaz PC, Burton TL, Burns SC, Hung JY, Penalva LO. Musashi1 modulates cell proliferation genes in the medulloblastoma cell line Daoy. BMC Cancer 2008; 8: 280.
55. Götte M, Wolf M, Staebler A, Buchweitz O, Kelsch R, Schüring AN, Kiesel L. Increased expression of the adult stem cell marker Musashi-1 in endometriosis and endometrial carcinoma. J Pathol 2008; 215: 317–29.
56. Yano H, Ohe N, Shinoda J, Yoshimura S, Iwama T. Immunohistochemical study concerning the origin of neurocytoma – a case report. Pathol Oncol Res 2009; 15: 301–5.
57. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, Lu L, Irvin D, Black KL, Yu JS. Analysis of gene expression and chemoresistance of $CD133^{+}$ cancer stem cells in glioblastoma. Mol Cancer 2006; 5: 67.
58. Ye F, Zhou C, Cheng Q, Shen J, Chen H. Stem-cell-abundant proteins Nanog, Nucleostemin and Musashi1 are highly expressed in malignant cervical epithelial cells. BMC Cancer 2008; 8: 108.
59. Sureban SM, May R, George RJ, Dieckgraefe BK, McLeod HL, Ramalingam S, Bishnupuri KS, Natarajan G, Anant S, Houchen CW. Knockdown of RNA binding protein musashi-1 leads to tumor regression in vivo. Gastroenterology 2008; 134: 1448–58.
60. Lovell MA, Markesbery WR. Ectopic expression of Musashi-1 in Alzheimer disease and Pick disease. J Neuropathol Exp Neurol 2005; 64: 675–80.
61. Ziabreva I, Perry E, Perry R, Minger SL, Ekonomou A, Przyborski S, Ballard C. Altered neurogenesis in Alzheimer's disease. J Psychosom Res 2006; 61: 311–6.
62. Oki K, Kaneko N, Kanki H, Imai T, Suzuki N, Sawamoto K, Okano H. Musashi1 as a marker of reactive astrocytes after transient focal brain ischemia. Neurosci Res 2010; 66: 390–5.
63. Crespel A, Rigau V, Coubes P, Rousset MC, de Bock F, Okano H, Baldy-Moulinier M, Bockaert J, Lerner-Natoli M. Increased number of neural progenitors in human temporal lobe epilepsy. Neurobiol Dis 2005; 19: 436–50.
64. Charlesworth A, Wilczynska A, Thampi P, Cox LL, MacNicol AM. Musashi regulates the temporal order of mRNA translation during Xenopus oocyte maturation. EMBO J 2006; 25: 2792–801.
65. Sagata N, Oskarsson M, Copeland T, Brumbaugh J, Vande Woude GF. Function of c-mos proto-oncogene product in meiotic maturation in Xenopus oocytes. Nature 1988; 335: 519–25.
66. Prasad CK, Mahadevan M, MacNicol MC, MacNicol AM. Mos 3′ UTR regulatory differences underlie species-specific temporal patterns of Mos mRNA cytoplasmic polyadenylation and translational recruitment during oocyte maturation. Mol Reprod Dev 2008; 75: 1258–68.
67. Battelli C, Nikopoulos GN, Mitchell JG, Verdi JM. The RNA-binding protein Musashi-1 regulates neural development through the translational repression of $p21^{WAF-1}$. Mol Cell Neurosci 2006; 31: 85–96.
68. de Sousa Abreu R, Sanchez-Diaz PC, Vogel C, Burns SC, Ko D, Burton TL, Vo DT, Chennasamudaram S, Le SY, Shapiro BA, Penalva LO. Genomic analyses of musashi1 downstream targets show a strong association with cancer-related processes. J Biol Chem 2009; 284: 12125–35.
69. Horisawa K, Imai T, Okano H, Yanagawa H. 3′-Untranslated region of doublecortin mRNA is a binding target of the Musashi1 RNA-binding protein. FEBS Lett 2009; 583: 2429–34.
70. Gleeson JG, Allen KM, Fox JW, Lamperti ED, Berkovic S, Scheffer I, Cooper EC, Dobyns WB, Minnerath SR, Ross ME, Walsh CA. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell 1998; 92: 63–72.
71. des Portes V, Pinard JM, Billuart P, Vinet MC, Koulakoff A, Carrié A, Gelot A, Dupuis E, Motte J, Berwald-Netter Y, Catala M, Kahn A, Beldjord C, Chelly J. A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. Cell 1998; 92: 51–61.
72. Sossey-Alaoui K, Hartung AJ, Guerrini R, Manchester DK, Posar A, Puche-Mira A, Andermann E, Dobyns WB, Srivastava AK. Human doublecortin (DCX) and the homologous gene in mouse encode a putative $Ca2^{+}$-dependent signaling protein which is mutated in human X-linked neuronal migration defects. Hum Mol Genet 1998; 7: 1327–32.
73. Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N, Bogdahn U, Winkler J, Kuhn HG, Aigner L. Doublecortin expression levels in adult brain reflect neurogenesis. Eur J Neurosci 2005; 21: 1–14.
74. Ocbina PJ, Dizon ML, Shin L, Szele FG. Doublecortin is necessary for the migration of adult subventricular zone cells from neurospheres. Mol Cell Neurosci 2006; 33: 126–35.
75. Glazer RI, Wang XY, Yuan H, Yin Y. Musashi1: a stem cell marker no longer in search of a function. Cell Cycle 2008; 7: 2635–9.
76. Ratti A, Fallini C, Cova L, Fantozzi R, Calzarossa C, Zennaro E, Pascale A, Quattrone A, Silani V. A role for the ELAV RNA-binding proteins in neural stem cells: stabilization of Msi1 mRNA. J Cell Sci 2006; 119: 1442–52.
77. Rigaut G, Shevchenko A, Rutz B, Wilm M, Mann M, Séraphin B. A generic protein purification method for protein complex characterization and proteome exploration. Nat Biotechnol 1999; 17: 1030–2.
78. Horisawa K, Tateyama S, Ishizaka M, Matsumura N, Takashima H, Miyamoto-Sato E, Doi N, Yanagawa H. In vitro selection of Jun-associated proteins using mRNA display. Nucleic Acids Res 2004; 32: e169.
79. Horisawa K, Doi N, Yanagawa H. Use of cDNA tiling arrays

for identifying protein interactions selected by in vitro display technologies. PLoS One 2008; 3: e1646.
80. Ghazal G, Ge D, Gervais-Bird J, Gagnon J, Abou Elela S. Genome-wide prediction and analysis of yeast RNase III-dependent snoRNA processing signals. Mol Cell Biol 2005; 25: 2981–94.
81. Ule J, Jensen KB, Ruggiu M, Mele A, Ule A, Darnell RB. CLIP identifies Nova-regulated RNA networks in the brain. Science 2003; 302: 1212–5.
82. Gold L, Brown D, He Y, Shtatland T, Singer BS, Wu Y. From oligonucleotide shapes to genomic SELEX: novel biological regulatory loops. Proc Natl Acad Sci USA 1997; 94: 59–64.