Published in final edited form as:

Biochem Soc Trans. 2008 June ; 36(Pt 3): 528–530. doi:10.1042/BST0360528.

Function and regulation of the mammalian Musashi mRNA translational regulator

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Abstract

The evolutionarily conserved RNA binding protein, Musashi, regulates neural stem cell self-renewal. Musashi expression is also indicative of stem cell populations in breast and intestinal tissue and is linked to stem cell over-proliferation in cancers of these tissues. Musashi has been primarily implicated as a repressor of target mRNAs in stem cell populations. However, little is known about the mechanism by which Musashi exerts mRNA translational control or how Musashi function is regulated. Recent findings in oocytes of the frog, *Xenopus*, indicate an unexpected role for Musashi as an activator of a number of maternal mRNAs during meiotic cell cycle progression. Given the importance of Musashi function in stem cell biology and the implications of aberrant Musashi expression in cancer, it is critical to understand the molecular processes that regulate Musashi function.

Musashi-mediated neural stem cell proliferation and cancer

Musashi is an RNA binding protein that regulates the translation of target mRNAs during neural development. Musashi was originally identified as a regulator of asymmetric stem cell division in *Drosophila* [1]. A subsequent mouse knockout of both Musashi-1 and the highly related Musashi-2 gene has revealed a critical contribution of Musashi for maintenance of mammalian adult neural stem cell populations [2]. Musashi expression has also been reported for a variety of tumor cells of neural and epithelial origin [3–9]. Musashi appears to function physiologically as a translational repressor of target mRNAs encoding cell cycle inhibitory proteins, thus maintaining neural stem cells in an undifferentiated state capable of self-renewal. Pathologically, up-regulation of Musashi may participate in cellular transformation through repression of target mRNAs involved in the inhibition of cell proliferation and commitment to differentiation.

In neural stem cells, Musashi acts to repress the translation of target mRNAs through sequence-specific interaction with the 3' UTR of the target mRNAs. Musashi has been shown to repress the translation of the mRNA encoding mammalian Numb, an inhibitor of Notch signaling and as a consequence induce up-regulation of Notch signaling [10]. Notch signaling is implicated as an important component of neural stem/progenitor cell proliferation [11–13]. In addition to Numb, Musashi has been shown to repress the translation of mRNA encoding the cell cycle inhibitor, p21WAF [14]. De-repression of p21WAF is necessary for the cell cycle arrest and commitment of proliferating neural progenitor cells to exit the cell cycle and undergo neuronal differentiation [15–17]. Recently, a novel function has been described in *Xenopus*, where

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Musashi-1 unexpectedly induces the translational activation of the Mos mRNA, as well as a number of other target mRNAs [18].

Mechanism of Musashi-mediated mRNA translational control

The mechanism by which Musashi exerts 3' UTR-directed mRNA translational control is not known. To determine if Musashi may act to inhibit 5' cap-dependent translational initiation, we utilized a series of luciferase reporter mRNAs that either had a 5' cap structure or a second series that lacked a 5' cap but instead contained an internal ribosome entry site (IRES) in the 5' UTR. Following transfection of the *in vitro* transcribed reporter mRNAs into the PC12 neural progenitor cell line, luciferase activity was assessed as an indicator of the efficacy of 3' UTR-directed translational control of the reporter mRNAs. We found that a 3' UTR containing a Musashi regulatory element was able to mediate repression of the reporter mRNA if a 5' cap was present, whereas Musashi interaction with the 3' UTR was unable to repress a reporter mRNA under IRES-dependent translational control (A. Wilczynska, M. C. MacNicol and A. M. MacNicol, unpublished data). These data demonstrate that Musashi interaction with a target mRNA 3' UTR exerts translational repression *via* a 5' cap-dependent mechanism.

Regulation of Musashi function

Musashi expression is down-regulated during neuronal differentiation and Musashi is not expressed in fully differentiated neuronal cell types [5,14]. However, early phases of differentiation of human neuroblastoma SH-SY5Y cells results in a HuD-mediated stabilization of the Musashi mRNA and an increase in Musashi protein [19]. Increased Musashi protein is similarly correlated with neural differentiation of P19 embryonic carcinoma cells, during the transition from proliferation to differentiation of neural stem and progenitor cells [14]. Similarly, we have found that Musashi protein persists for at least 6 days during neural differentiation of primary rat stem/progenitor cells and PC12 cells, by which time the cells have formed elaborate neurite extensions (A. Wilczynska, M. C. MacNicol and A. M. MacNicol, unpublished data). These findings suggest that the ability of Musashi to mediate repression must be attenuated during differentiation, since target mRNA translation is necessary for neural differentiation. The regulatory processes governing de-repression of Musashi-mediated mRNA translation are thus a key focus for future studies.

Musashi subcellular localization

Given the clear requirement for Musashi in regulating target mRNA translation in the cytoplasm, there is much ambiguity as to the precise subcellular localization of the protein [20]. Musashi has reported to be exclusively or predominantly cytoplasmic, diffuse throughout the cell, nuclear or localized in perinuclear foci depending on the cell type. To better understand the subcellular localization of Musashi in a neuronal progenitor cell line, we utilized a GFP-tagged form of full length mammalian Musashi-1 in proliferating and differentiating PC12 cells. Musashi protein was found to be heterogeneously distributed between the cytoplasmic and nuclear compartments in proliferating populations of PC12 cells. Interestingly, upon neuronal differentiation, Musashi was redistributed toward the nuclear compartment suggesting nuclear sequestration as a mechanism to de-repress cytoplasmic target mRNAs (A. Wilczynska, M. C. MacNicol and A. M. MacNicol, unpublished data). We have identified and characterized a nuclear localization signal (NLS) within the first RNA-binding motif (RRM1) of the mammalian Musashi-1 protein. The NLS is also conserved in the related Musashi-2 protein. Interestingly, RRM1 is the critical determinant of Musashi RNA binding specificity [21] and so interaction with target mRNAs in the cytoplasm may occlude the NLS. In the nucleus, RNA binding activity facilitates nuclear export of the Musashi protein. Furthermore, an additional domain within the N-terminal region of the protein is also required for efficient

nuclear export, suggesting that nuclear export of Musashi may be a consequence of two cooperative mechanisms (A. Wilczynska, M. C. MacNicol and A. M. MacNicol, unpublished data). The regulatory mechanisms that switch the import/export balance to promote Musashi nuclear localization during differentiation remain to be determined.

Conclusions

Musashi exerts translational repression of target mRNAs *via* a 5' cap dependent mechanism in neural stem cells, but paradoxically exerts target mRNA translational activation during meiotic cell cycle progression in *Xenopus* oocytes. While Musashi promotes cell cycle progression in both cases, a key future goal will be to elucidate the cell type-dependent and/or context dependent regulatory factors that can determine whether Musashi exerts repression or translational activation. Musashi subcellular localization appears to be regulated during neuronal differentiation of stem/progenitor cell populations. It will be interesting to determine if nuclear sequestration of Musashi facilitates cytoplasmic target mRNA translation or if Musashi-1 has distinct nuclear functions, such as alternative splicing of certain pre-mRNAs [22].

Acknowledgements

This work was supported by ACS RPG 101279 and NIH RO1 HD35688 to A.M.M., and NIH grant RR020146 to M.C.M. Due to space constraints, we apologize to any of our colleagues we failed to cite in the manuscript.

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