Identification of nuclear export mechanisms of myosin IC

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Abstract

Myosin IC (MyoIC) is a member of the myosin superfamily that plays an important role in dynamic nuclear processes. Understanding the mechanisms that contribute to the nucleo-cytoplasmic transport of MyoIC will provide valuable insights into its nuclear function regulation. The objective of this study was to identify the nuclear export signal (NES) of MyoIC. To this effect, I created various MyoIC-GFP expression constructs with deletions or mutations in specific amino acids using site directed mutagenesis. The cellular localization of the GFP-fusion proteins was analyzed through fluorescence microscopy of transfected mammalian cells. Cells expressing various constructs were treated with Leptomycin B, a known pharmacological NES inhibitor and analyzed for changes in the cellular localization of the respective constructs. Results from these experiments suggest the presence of an NES in tail region of MyoIC. These data are an important first step in identifying the pathways and factors that contribute to the nuclear localization of MyoIC.

Introduction

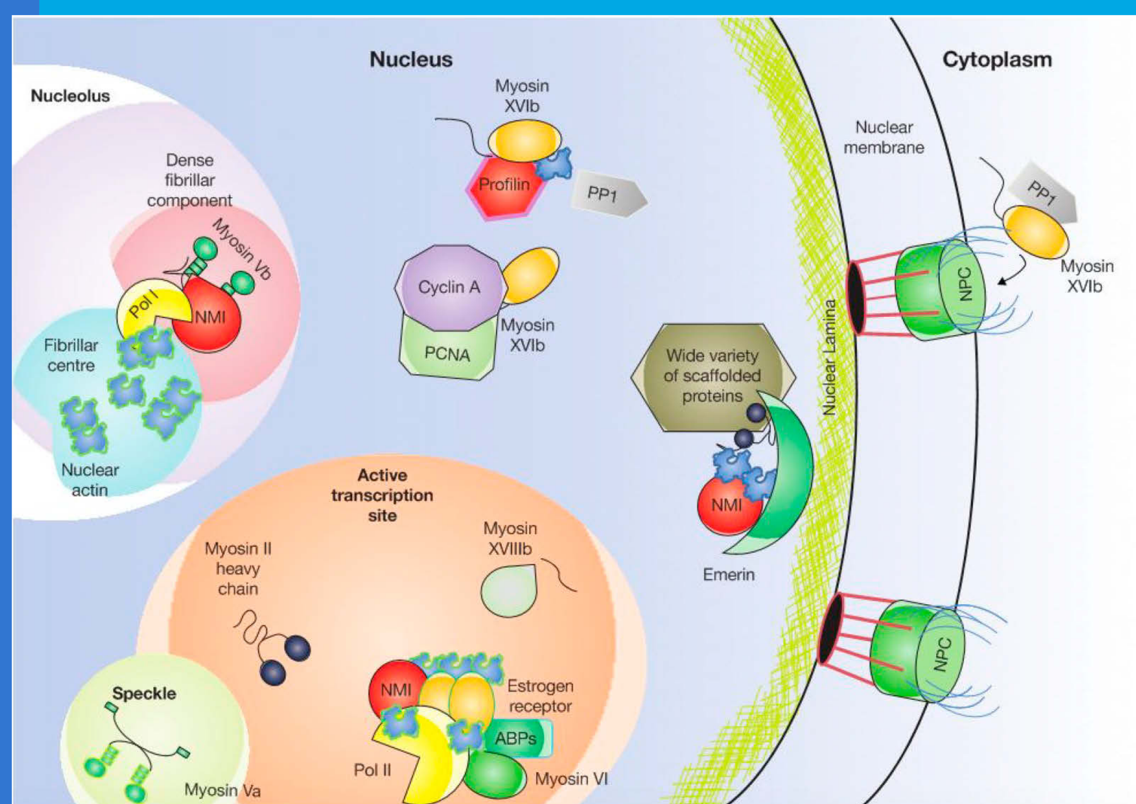


Figure 1. Nuclear MyoIC is functionally involved in transcription by RNA polymerases I and II and in various intranuclear transport processes [1,2].

- The presence of MyoIC in the nucleus is well established and nuclear functions have been identified (Fig. 1).
- However, little is known about the regulation of nuclear MyoIC functions.
- The **aim** of this study is to identify the mechanisms that are involved in the nuclear export of MyoIC.
- The **rationale** is that understanding how MyoIC gets in and out of the nucleus will likely contribute to our understanding of the regulation of nuclear processes that MyoIC is involved in.

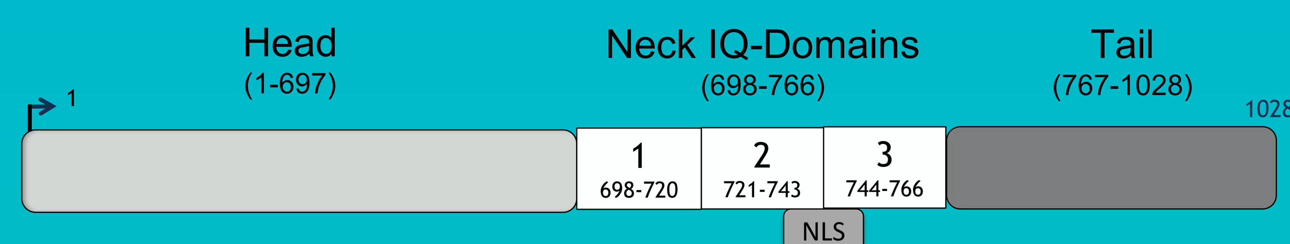


Figure 2. Schematic representation of MyoIC structure, including the nuclear localization signal (NLS) [3].

Methods

1) Induction of MyoIC translocation

- Transfection of PC-3 cells grown on coverslips with MyoIC-EGFP constructs
- 24hr after transfection: treatment of cells with 2μM ionomycin or DMSO (control) for 10 min at 37°C
  - ➢ Ionomycin: a ionophore that transports Ca^{2+} ions across membranes and induces an increase in intracellular Ca^{2+} levels
- Removal of ionomycin and treatment with 20 nM LMB or EtOH (control) for 1 hour at 37°C
  - ➢ LMB: a specific inhibitor of CRM1-mediated nuclear export
- After each step, cells were fixed and prepared for microscopic analysis of fluorescent MyoIC-EGFP constructs

2) Analysis of MyoIC cellular distribution by fluorescence microscopy

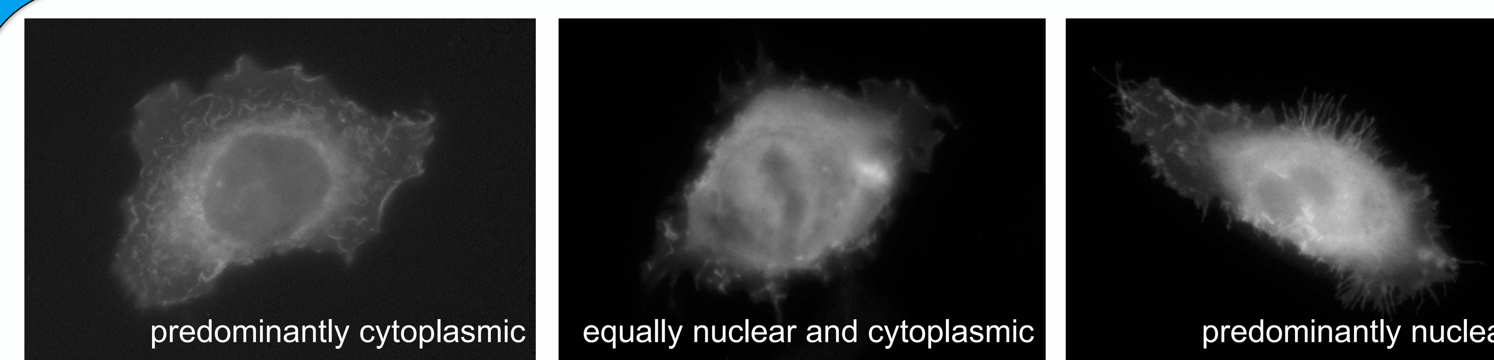


Figure 3. Images represent the various nucleocytoplasmic localizations of MyoIC-EGFP within the PC-3 cell population. After each experiment, images of at least 100 cells were taken and scored into one of the indicated categories to detect changes in intracellular distribution of MyoIC.

Results

Figure 4. Intracellular calcium increase causes nuclear import of MyoIC

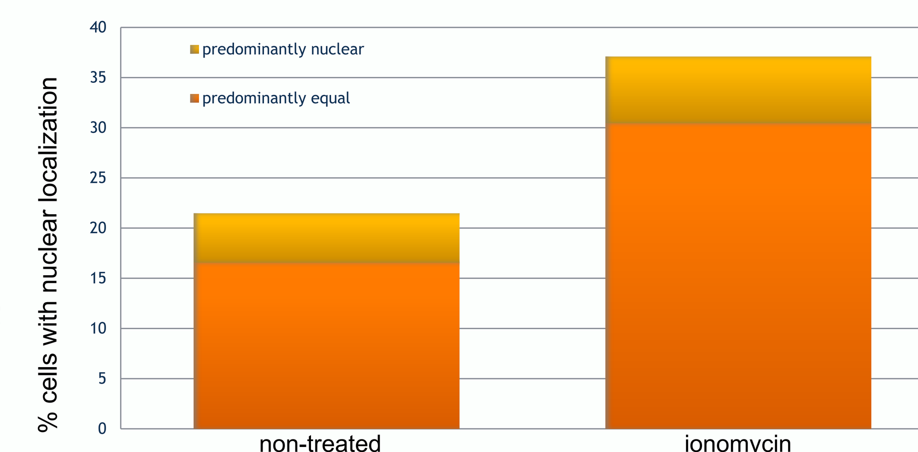


Figure 5. Removal of ionomycin facilitates active nuclear export

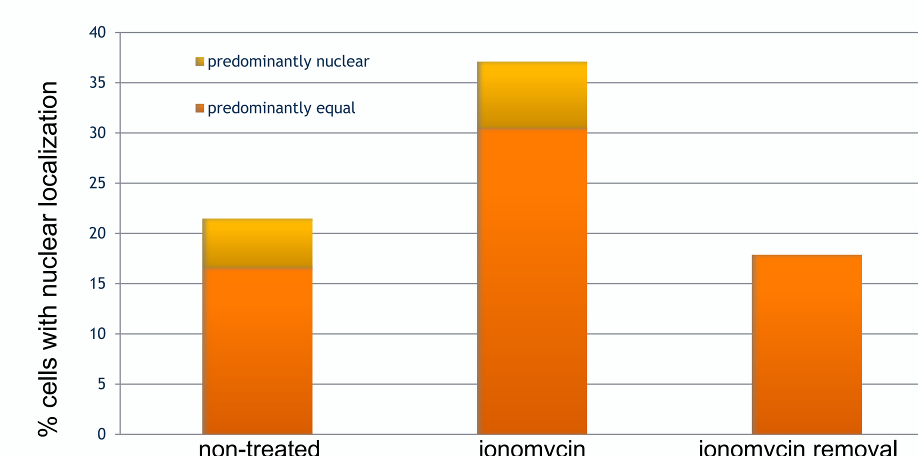
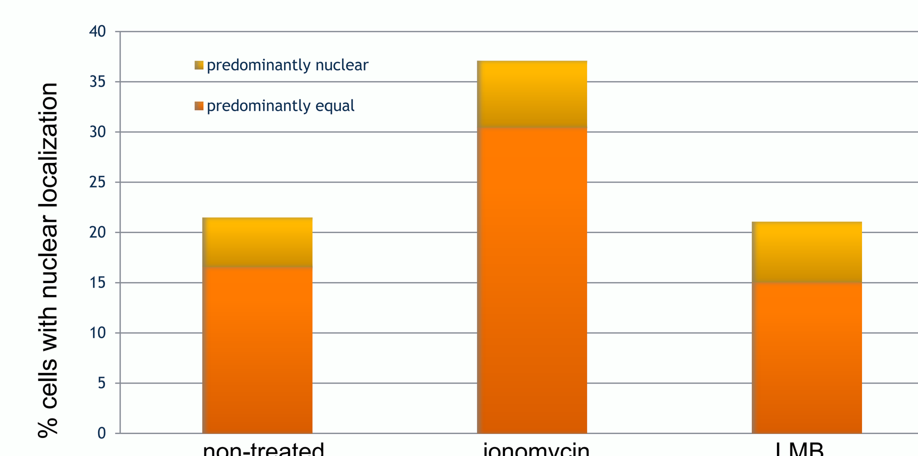


Figure 6. LMB does not inhibit nuclear export of MyoIC



Leptomycin B (LMB) = CRM1 specific export inhibitor

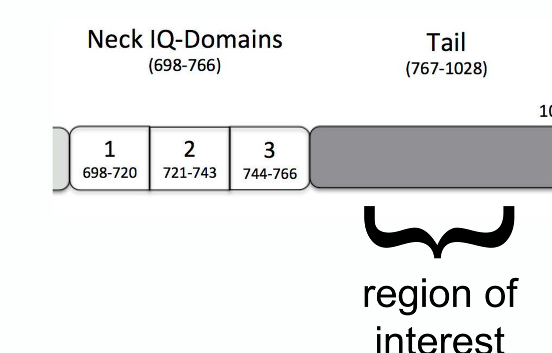
Conclusions

- Ionomycin treatment induces nuclear import of MyoIC → import is calcium dependent
- Removal of ionomycin facilitates active nuclear export of MyoIC
- LMB does not inhibit nuclear export of MyoIC after ionomycin removal → export is not facilitated by the export factor CRM1
- Calcium changes induced by ionomycin serve as effective system to analyze import and export modifications of MyoIC

Future Directions

Use MyoIC calcium dependent translocation system to identify the localization of NES of MyoIC by:

- Establishing timeline of nuclear export according to export model
  - ➢ Will provide information about the efficiency of export
- Utilize identified potential NES sites in the MyoIC tail domain to determine effects of altered MyoIC constructs within export model
  - ➢ Will identify the exact localization and composition of the NES



(767) C P E N A F F L D H V R T S
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H L A V V A P R L N S R (1028)

Figure 7. Leucine-rich regions characteristic of potential NES of MyoIC [4]: AA 905-907, AA 1008-1010

References and Acknowledgements

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We would like to thank Ivan V. Maly for assistance with the methods and procedure. This study was funded in part by an Undergraduate Research Award from the University at Buffalo's Center for Undergraduate Research and Creative Activities (CURCA) to V.G.