Identification of nuclear export mechanisms of myosin IC

Victoria A. Gosy
Faculty Mentor, Wilma A. Hofmann
Department of Physiology and Biophysics
The University at Buffalo, Buffalo, NY
Presence of MyoIC in nucleus well established; functions have been identified (Hofmann et al., Biochem Cell Bio 2006)

Little known about regulation of function

Nuclear functions of MyoIC (de Lanerolle & Serebryannyy, Nat Cell Bio 2011)
Myosin IC structure

- **Head** (1-697)
- **Neck IQ-Domains** (698-766)
- **Tail** (767-1028)

1. 1 598-720
2. 2 721-743
3. 3 744-766

(NLS)

(Schwab et al., Exp Cell Res 2013)
Research Question

What mechanisms contribute to regulation of the nuclear export of myosin IC?
Methods

1. Transfection of mammalian prostate cancer cells (PC-3) with MyoIC-EGFP construct
2. Experimental conditions
3. Cells fixed and prepared for microscopic analysis of MyoIC-EGFP
4. Analysis with fluorescence microscopy via scoring of MyoIC distribution

Various nucleocytoplasmic localizations used in scoring to detect changes in intracellular distribution of MyoIC.
Baseline MyoIC distribution

MyoIC distribution in PC-3 cells

% cells with nuclear localization

- predominantly nuclear
- predominantly equal

predominantly cytoplasmic
equally nuclear, cytoplasmic
predominantly nuclear
Intracellular calcium increase causes nuclear import of MyoIC

+ Treatment of PC-3 cells with 2 μM ionomycin or DMSO (control)
+ Ionomycin: ionophore that transports Ca^{2+} across membrane and increases intracellular Ca^{2+} levels
Removal of ionomycin facilitates active nuclear export
LMB does not inhibit nuclear export of MyoIC

- Treatment of PC-3 cells with 20 nM Leptomycin B (LMB) or EtOH (control)
- LMB: inhibitor of CRM-1 specific nuclear export

![Bar chart showing percentage of cells with nuclear localization after different treatments.]

- **Non-treated**
  - Predominantly nuclear: ~20%
  - Predominantly equal: ~10%
  - Total: ~30%

- **Ionomycin treated**
  - Predominantly nuclear: ~35%
  - Predominantly equal: ~5%
  - Total: ~40%

- **Leptomycin B**
  - Predominantly nuclear: ~25%
  - Predominantly equal: ~15%
  - Total: ~40%
Conclusions

- Ionomycin treatment induces nuclear import of MyoIC \( \rightarrow \) import is Ca\(^{2+}\) dependent
- Removal of ionomycin facilitates active nuclear export of MyoIC
- LMB does not inhibit nuclear export of MyoIC after ionomycin removal \( \rightarrow \) export not facilitated by export factor CRM-1
- Ca\(^{2+}\) changes induced by ionomycin serve as effective system to analyze import and export modifications of MyoIC
Future Directions

+ Use MyoIC Ca\(^{2+}\) dependent export model to identify the localization of **nuclear export signal** of MyoIC by:
  + Establishing timeline of nuclear export according to export model (information on export efficiency)
  + Utilizing identified potential NES sites to determine effects of altered MyoIC constructs within model

Leucine-rich regions characteristic of potential NES of MyoIC: AA 905-907, AA 1008-1010 (CBSA, NetNES 1.1 Server)
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Questions?