

Nucleocytoplasmic Transport

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Urocortin II Regulates NFAT Transcription Factor in Adult Rabbit Cardiac Myocytes

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Nuclear factor of activated T cells (NFAT) transcription factors play a key role during cellular remodelling associated with hypertrophy and heart failure (HF). We tested the hypothesis that the cardioprotective peptide Urocortin II (UcnII) regulates translocation and activation of NFAT.

Rabbit ventricular myocytes were infected with recombinant adenoviruses encoding for NFAT-GFP fusion proteins (isoforms NFATc1 and NFATc3). The subcellular distribution of NFAT was quantified as the ratio of NFAT_{nuc} to NFAT_{cyt} fluorescence (R_{NFAT}) and nuclear-cytosolic NFAT translocation was expressed as changes of R_{NFAT} . Under basal unstimulated conditions NFATc3 was predominantly localized in the cytoplasm, whereas NFATc1 displayed a nuclear localization. UcnII (100 nM) caused NFATc1 export to the cytoplasm (R_{NFAT} decreased from 2.25 to 1.03 after 24 hrs). UcnII did not affect nuclear-cytosolic NFATc3 distribution.

UcnII-induced NFATc1 export to the cytoplasm was significantly ($P < 0.05$) reduced (by 58-79%) by inhibition of guanylate cyclase (ODQ, 10 μ M), calcineurin (Cyclosporin A, 1 μ M), and GSK3 β (Alsterpaullone, 1 μ M). Inhibition of PI3K (Wortmannin, 300 nM) and nuclear transport protein crm1 (Leptomycin B, 40 nM) prevented the redistribution of NFATc1 to the cytoplasm completely.

In ventricular myocytes from failing rabbit hearts, nuclear localization of NFATc3 was significantly enhanced compared to normal myocytes (R_{NFAT} 0.78 vs. 0.58). Stimulation of HF myocytes with UcnII resulted in a translocation of NFATc3 back to the cytoplasm (R_{NFAT} =0.56). In HF myocytes the nuclear localization of NFATc1 was less pronounced compared to normal myocytes (R_{NFAT} : 1.97 in HF vs. 2.86 in normal ventricular cells). UcnII induced a further redistribution of NFATc1 out of the nucleus (R_{NFAT} =0.97).

We conclude that UcnII enhances the export of NFAT from the nucleus to the cytoplasm in ventricular myocytes involving the PI3K/cGMP/PKG/calcineurin- and PI3K/Akt/GSK3 β -pathways and could thus positively affect remodeling in hypertrophy and HF.

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Binding to FG-Nups Is Insufficient to Promote Nuclear Transport

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Nuclear pore complexes (NPCs) mediate all cargo traffic between the nucleus and the cytoplasm of eukaryotic cells. The central channel is occupied by a network of intrinsically disordered polypeptides that contain thousands of phenylalanine-glycine (FG) repeats. Transport factors bind to the FG-network and carry cargos through the NPC. The precise mechanism by which transport factors allow cargos to overcome and migrate through the permeability barrier is unknown. Single particle tracking experiments were used to determine the transport properties of a large cargo, the tetrameric protein beta-galactosidase (~500 kDa), with four M9 signal sequences (M9- β Gal). With 1 μ M transportin and 100 pM M9- β Gal, the cargo transport efficiency was $24 \pm 4\%$ and the NPC interaction time was 8.7 ± 0.8 ms. When the transportin concentration was reduced to 25 pM, these parameters were $3 \pm 2\%$ and 4.8 ± 0.4 ms, respectively. These data indicate that multiple transportin molecules are required for efficient transport and a single transport factor allows binding but not transport. Particle tracking data indicate a central barrier to translocation, and therefore support the hypothesis that transport factors promote translocation by increasing cargo solubility within the central FG-network. The weak dependence of interaction time on the number of bound transport cofactors indicates that avidity effects are low, implying both a low effective concentration of free FG repeats and a millimolar FG-transportin affinity. A weak FG-transportin affinity implies that any FG-FG interactions must also be weak, otherwise transport receptor interactions would be insufficient to overcome the permeability barrier. These results are consistent with a model in which the permeability barrier is comprised of a denser central FG-network and cargo recognition occurs within a sparser peripheral FG-network.

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Effect of FG Motifs on Ordering Proteins within the Nuclear Pore Complex

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Transport across the nuclear membrane occurs through the nuclear pore complex (NPC). The transport through the NPC is both selective and rapid, and involves the phenylalanine glycine repeat nucleoporins (FG nups). The FG motifs can bind cargo and may also be able to interact with each other but the mechanism of transport remains unclear. We have developed a theoretical framework which exploits the symmetry of the NPC and its organization in the nuclear envelope to resolve the order and disorder of individual protein domains within the complex. Specific domains of individual nucleoporins (nups) were tagged with GFP and examined using fluorescence polarization microscopy to determine their organization and dynamics. We characterized the degree to which the FG domains are ordered in vivo. This approach revealed different degrees of organization within a single FG domain: the tips and middle of the FG domains are less ordered than NPC-anchored domains, but still display a surprising degree of organization. We examined three mammalian nups: Nup54, Nup62, and Nup98. We observe that of these, Nup54 is the most ordered within the NPC and Nup62 is the least ordered. We then investigated the contribution of the FG repeats to the organization of these nucleoporins by mutating the FG motifs to AG. We show that these mutations have no effect on the organization of Nup62, decrease but do not eliminate the order shown by Nup54, and prevent Nup98 from localizing correctly to the NPC. We therefore propose that multiple factors contribute to the organization of FG nups in vivo, and that the influence of the FG repeats on order varies between different FG nups.

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Nuclear Export of Single Native mRNA

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Export of nuclear mRNA is a key transport process in eukaryotic cells, but its dynamics and regulation is not well understood. We labelled native mRNA particles in living *Chironomus tentans* salivary gland cells [1, 2] using fluorescent hrp36, the *C. tentans* homolog of mammalian hnRNP A1, and the nuclear envelope by fluorescent NTF2. Using light sheet microscopy we succeeded to image single mRNA particles more than 100 micrometer deep in the sample [3] and traced for the first time single native mRNA particles across the nuclear envelope. The particles often probed nuclear pore complexes at their nuclear face with an average duration of $\tau \sim 60$ ms, but the actual export process was about ten-fold longer ($\tau \approx 0.54$ s) containing a distinct rate-limiting step. Analysis of single fluorescent Dbp5 proteins, the RNA helicase essential for mRNA export, revealed that Dbp5 was exclusively encountered at the cytoplasmic face of the nuclear pore complex with a binding duration of $\tau \sim 50$ ms. Our results suggest that single mRNA particles required >10 cycles of remodelling by Dbp5 to accomplish export directionality.

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[2] Veith, R., T. Sorkalla, E. Baumgart, J. Anzt, H. Häberlein, S. Tyagi, J. P. Siebrasse, and U. Kubitscheck. 2010. Biophys. J. 99: 2676-2685.

[3] Ritter, J.G., J. P. Siebrasse, R. Veith, A. Veenendaal, and U. Kubitscheck. 2010. PLoS ONE 5, e11639.

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Three-Dimensional Characterization of the Selective Barrier formed by Unfolded Proteins in the Nuclear Pore Complex

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The nuclear pore complexes (NPCs) mediate the bidirectional trafficking of macromolecules between the cytoplasm and the nucleus in eukaryotic cells. The selective barrier formed by natively unfolded phenylalanine-glycine (FG) nucleoporins (Nups) inside the NPC allows for passive and facilitated transport through the NPC. However, the mechanism of formation and spatial distribution of FG-barrier in the NPC remains unresolved. By a newly developed single-molecule microscopy, single-point edge-excitation subdiffraction (SPEED) microscopy, we have used various fluorescent transport