# Functional adaptations revealed by mapping of communication pathways in the processive motor myosin 5a

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**Theses**

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INTRODUCTION

Myosins convert the chemical energy stored in ATP into mechanical work, thereby driving various eukaryotic motile systems e.g. muscle contraction, tension maintenance and intracellular cargo transport.

Mechanochemical energy transduction is realized by the cyclic actomyosin interaction. The actomyosin cyle consists of similar enzymatic steps in different myosins performing widely varying functions in cells. However, the fascinating functional diversity of the myosin superfamily requires specific and/or unique adaptations of the actomyosin cycle of different myosins to the specific function. Functional adaptations of myosin motors, besides the alterations of domain structure, are attributed to class- and isoform-specific mechanisms encoded within the motor domain (MD). The MD is considered as the „central control unit" of myosins. In all myosins the MD contains regions responsible for the binding of the actin track and ATP. The MD also comprises the converter-region, which forms the basis of the lever.

Nevertheless, the adaptation mechanisms of the MD and many of the underlying structural rearrangements are not understood in detail.

Myosin 5a is an intracellular transporter motor that moves processively[1] along the actin filament by a hand-over-hand mechanism. It participates in melanocyte transport and in *SER* transport into the dendritic spines of *Purkinje*-cells, and also in signal transduction of the retina and *cochlea* receptor cells.

Due to the high complexity of the processive hand-over-hand stepping mechanism of myosin 5a on the actin filament, myosin 5a assumes and requires unique adaptation mechanisms in the actomyosin cycle of myosin heads. In my PhD work I aimed at the mapping of these functional adaptation mechanisms of the intracellular cargo transporter myosin 5a. We altered the wild-type inter - and intramolecular communication pathways by introducing point mutations into the functional regions of the MD (nucleotide binding site; N-terminal subdomain (NTS) which communicates with *converter* during the actomyosin cycle).

Mapping of adaptation mechanisms in the background of complex working mechanism of myosin 5a does not only contribute to the understanding of myosin 5a properties but also may provide insight into how the subtle structural adaptations of the common structural scaffold makes myosin motors enable to satisfy widely different physiological demands.

[1] processive: capable of taking several enzymatic cycles
and coupled mechanical steps without the detachment from the track during one run

AIMS

Our aim was to map novel mechanisms underlying the complex processive working mechanism of myosin 5a and to point out how myosins with different functions are capable of tuning structural rearrangements of the common structural scaffold to the specific functions.

*Aim of first topic:*

To reveal the role of myosin-class specific residue (**X**) of *switch-2* (LDI**X**GFE) in the nucleotide-binding pocket during the mechanochemical transduction of myosin 5a (myosin 5a possesses tyrosine (Y439) in the variable position of *switch-2* (LDI**Y**GFE))

*Aim of second topic:*

To identify the contributions of N-terminal subdomain (NTS) - *converter* interface in the actomyosin enzyme cycle by mimicking a repulsive interaction (K84-R704, *D. discoideum*) of myosin 2 motor in myosin 5a (I67K-R709).

- We fine-tuned the rearrangements of myosin 5a motor domain during the actomyosin cycle by point mutations.
- We characterized the whole actomyosin ATPase cycle by dissecting the individual steps of the enzyme cycle.
- We identified the effect of point mutations on the motility of myosin 5a.
- We identified and validated the structural rearrangements in the background of kinetic and motility results by structural and kinetic modeling methods.

APPLIED TECHNIQUES

- We produced one-headed (S1, subfragment-1) and two-headed (HMM, heavy meromyosin) wild-type and point mutant DNA constructs of mouse (*Mus musculus*) myosin 5a by *QuikChange* mutagenesis.

  First topic: *switch-2* mutants - Y439A, Y439S, Y439E S1 and Y439A HMM

  Second topic: *NTS* mutants - I67K S1 and HMM

- Production of recombinant myosin 5a proteins in baculovirus - *Sf*9 (*Spodoptera frugiperda*) expression system.
- Optimization and analysis of myosin 5a expression by SDS-PAGE/Western Blot method.

- Protein purification:

  Affinity chromatography – Flag affinity resins (myosin 5a (m5a) S1 and HMM)

  Ion exchange chromatography – Q Sepharose FF (m5a S1 and HMM)

  Ion exchange chromatography – Q Sepharose FF (PBP (Phosphate binding protein); MDCC (7-(diethylamino)-3-((((2-Malemidyl)ethyl)amino)carbonyl)coumarin – PBP)

- Modification of proteins:

  F-actin labeling on $Cys^{374}$ with N-(1-pyrene)iodoacetamide (PIA)

  Phosphate binding protein (PBP) labeling on $Cys^{197}$ with MDCC

- *Steady-state* ATPase activity (basal and actin-activated) by NADH - coupled assay (Shimadzu UV 2101PC spectrophotometer)
- *Steady-state* cosedimentation assays (Beckman L7-65 ultracentrifuge; GelQuant Pro, Bio-Imaging System)
- *Steady-state* fluorescence titration – following pyrene-actin fluorescence (SPEX Fluoromax spectrofluorometer)
- Tryptophan (Trp) fluorescence spectral changes (apo state, in the presence of nucleotides and nucleotide analogs)
- Rapid kinetic measurements:
  - KinTek SF-2004 and BioLogic SFM 300 *stopped-flow*

    Fluorescence signals:

    - Trp - excitation: 280 nm, 340 IF (Interference) filter
    - PYA - excitation: 365 nm, 400 LP (Long Pass) filter
    - md-ADP/md-ATP (3'-(N-methylantraniloyl)-2'-deoxy–ADP/ATP - excitation: 280 nm, 420 LP filter
    - MDCC-PBP - excitation: 436 nm, 455 LP filter

    Light scattering - excitation: 340 nm, 340 IF filter

  - KinTek RQF-3 *quenched-flow*
    - Radioisotope-labelled [$\gamma$-$^{32}$P-]ATP

- Motility measurements:
    - *Actin gliding assay* (OLYMPUS IX70 Microscope)
    - Single molecule motility *TIRF* - (*Total Internal Reflection Fluorescence*) Microscopy (OLYMPUS IX70)
- Structural modeling (MOE and Yale Morph Server)
- Global and *steady-state* kinetic modeling (KinTek Explorer 3.0 software)

RESULTS (THESES)

*Theses of first topic:*

- We characterized the kinetic properties of mouse (*Mus musculus*) myosin 5a actomyosin cycle.
- We revealed the regulatory role of the variable position of the *switch-2* nucleotide sensor in the actin-activated $Mg^{2+}$ - dependent ADP release process.
- We determined that the bulky tyrosine of myosin 5a *switch-2* enables rapid processive translocation of myosin 5a by inducing unique structure of *switch-2.*
- We showed evidence that ADP release directly determines the sliding speed of myosin 5a.
- We produced significantly slowed processive myosin 5a motors, indicating the possibility of separate modification of sliding speed.
- Furthermore, we identified that the bulky tyrosine of myosin 5a *switch-2* enables low energy-barrier *rigor* actin binding of myosin 5a by inducing a unique structure of *switch-2.*
- General regulatory role of the conserved *switch-2* in the actin- (or partner-) activated $Mg^{2+}$ - dependent nucleotide release process may be present in all nucleoside-triphosphatases (NTPases).

*Theses of second topic:*

- The perturbation of the functioning of the NTS-*converter* interface is coupled to alterations in the actin- and ATP-binding sites:
    - weakening of strong actomyosin interaction
    - abolishment of actin-activation of ADP release
    - emergence of a novel ATP-binding *off-pathway*[2] intermediate

[2] off-pathway: outside the main-flux pathway of enzyme cycle

- We produced slow but processive myosin 5a motors which are highly sensitive to mechanical drag and ATP concentration.
- By the modification of the NTS-*converter* interface we probably induced the alteration of a communication pathway that influences the mechanical load dependence of myosin 5a.
- We determined that the NTS-*converter* interface contributes to actomyosin cycle in different ways in different myosins.

CONCLUSIONS

By the perturbation of communication pathways in the motor domain we obtained comprehensive insights into the nucleotide exchange process of myosins and the coupled motile speed optimization strategy. Furthermore, we elucidated the mechanism of *rigor* actin binding in myosin 5a. We identified a mechanical load sensitive part of the myosin 5a motor domain, and induced the emergence of a novel ATP-binding *off-pathway* intermediate during the myosin mechanochemical cycle.

Our results revealed that structural rearrangements underlying functional adaptations provide the possibility of independent tuning of functional parameters (processivity, motile speed, load sensitivity). Nevertheless, the coupling of these communication pathways is observable in many cases during the energy-transducing actomyosin cycle.

Manipulation of processivity and/or translocation speed by functionally well-characterized point mutations may help the identification of the precise physiological roles of motor enzymes, e.g. reveal the role of myosin 5a in the signal transduction processes of photoreceptor cells. The manipulation of mechanical load sensitivity may contribute to the determination of mechanical exposure of myosin motors in physiological conditions.

## PUBLICATIONS CONCERNING THE THESES

### Scientific Publications

**Nagy, N. T.**, Chakraborty, S., Harami, G. M., Sellers, J. R., Sakamoto, T., Kovács, M. (2013): *A subdomain interaction at the base of the lever allosterically tunes the mechanochemical mechanism of myosin 5a.* **PLoS ONE** 2013 May 1;8(5):e62640.

**Nagy, N. T.**, Sakamoto, T., Takács, B., Gyimesi, M., Hazai, E., Bikádi, Z., Sellers, J. R., Kovács, M. (2010): *Functional adaptation of the switch-2 nucleotide sensor enables rapid processive translocation by myosin-5.* **FASEB J**. 2010 Nov;24(11):4480-90.

### Connected Hungarian Scientific Publications

**Nagy, N.**, Takács, B., Kovács, M. (2010): *Motorenzimek működési alapelvei és egyedi finomhangolása.* (Underlying principles of enzymatic mechanism and functional adaptation of molecular motors) ***Biokémia*** - online paper of Hungarian Biochemical Society

**Conference proceedings** (author – presenter is underlined)

**Nagy, N. T.**, Chakraborty, S., Sakamoto, T., Kovács, M. (2011): *Allosteric tuning of myosin 5a motor activity.* Europen Muscle Conference, Berlin, Germany

**Nagy, N. T.**, Kovács, M. (2011): *Allosteric tuning of myosin 5a motor activity.* 55th Annual Meeting of the Biophysical Society, Baltimore, MD, USA

**Nagy, N.**, Sakamoto, T., Takács, B., Gyimesi, M., Sellers, J. R., Kovács, M. (2009): *A class-specific structural adaptation of the switch-2 loop enables rapid processive translocation of myosin 5a.* European Muscle Conference, Lille, France

**Nagy, N.**, Sellers, J. R., and Kovács, M. (2008): *Role of the switch-2 active site loop in the processive mechanism of myosin 5.* 52nd Annual Meeting of the Biophysical Society, Long Beach, CA, USA

**Nagy, N.**, Sarlós, K., Takács, B., Tóth, J., Yang, Y., Pearson, D. S., Hetényi, C., Nyitray, L., Málnási-Csizmadia, A., Geeves, M. A., Bagshaw, C. R., Sellers, J. R., Brown, J. H., Szent-Györgyi, A. G., Cohen, C., Kovács, M. (2008): *Routes of allosteric communication between functional parts of the myosin motor.* Scientific Meeting of International Research Scholars of the Howard Hughes Medical Institute, Lisbon, Portugal

**Nagy, N.**, Kovács, M. (2007): *Role of the switch-2 active site loop in the processive mechanism of myosin 5.* Molecular Recognition Conference*, Pécs, Hungary*

**Nagy, N.**, Kovács, M. (2007): *Role of the switch-2 active site loop in the processive mechanism of myosin 5.* Annual Meeting of the Hungarian Biochemical Society, Debrecen, Hungary