1. ABSTRACT

Myosins are proteins that convert chemical energy stored in ATP into mechanical force that is applied on an actin filament. Nuclear myosin 1 (NM1) was the first myosin detected in the cell nucleus. Together with nuclear actin they were shown to play important roles in DNA transcription and chromatin remodeling. However, the molecular details of the NM1 functions are largely unknown. To expand our knowledge about this molecular motor we studied tissue expression, mechanism of nuclear localization and molecular interactions of this myosin motor.

In the first part we examined the expression pattern of NM1 in various mouse tissues. We demonstrated that NM1 is present in cell nuclei of all mouse tissues examined except for cells in terminal stages of spermatogenesis. Quantitative PCR and western blots demonstrated that the expression of NM1 in tissues varies, with the highest levels in the lungs. NM1 is a nuclear isoform of earlier identified myosin 1c (Myo1c), which was described initially as a cytosolic, and plasma membrane associated protein. The only known difference between these two proteins was the presence of additional 16 amino acids at the N-terminus of NM1. Next we focused on the influence of NM1 domains, including the N-terminus, on the subcellular localization of this protein. We found out that N-terminus is not required for nuclear entry. Surprisingly, we found a nuclear localization sequence placed within calmodulin-binding motif of NM1. This sequence is also present in the “cytoplasmic” Myo1c protein. We confirmed the presence of both isoforms in the nucleus. The notion that overexpressed NM1 and Myo1c could localize to the cell nucleus prompted us to inspect their colocalization in cells and tissues. Using polyclonal antibodies toward the N-terminus of NM1 and a monoclonal antibody against the tail domain we found that endogenous NM1 and Myo1c colocalize to a high extend in nucleus and at the plasma membrane. Finally, we looked for new interaction partners of NM1 using co-immunoprecipitation. We found set of actin- and phospholipid- binding proteins as the proteins that co-purified from the cell extracts with NM1. Interestingly, some of the identified proteins were also found in complex with Myo1c. We hypothesized that NM1 and Myo1c could be functionally similar transcript variants of the same gene.

The most intriguing result is that NM1 is more similar to Myo1c than previously imagined. Therefore one of the most interesting future questions would be to verify whether NM1 and Myo1c are functionally identical. Naturally, this requires further experiments.