Phosphorylated myosin Va localizes to nuclear speckles

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Nuclear actin and actin-associated proteins, including a nuclear isoform of myosin Iß, have recently been implicated in gene expression in eukaryotic cells. Myosin Va (MVa) is a processive, actin-based molecular motor involved in several vital cellular processes, including vesicle transport and anchorage, spindle pole alignment and RNA translocation. We have identified a phosphorylated form of MVa (ppMVa) in the nucleus of several types of cultured cells by using monoclonal antibodies generated against a synthetic phosphopeptide corresponding to Ser1650 and flanking regions in the globular tail domain of murine MVa. The antibody 9E6 recognized ppMVa in cell extracts by western blots and immunoprecipitated it from purified nuclear fractions of HeLa cells and B16-F10 melanocytes. Confocal microscopy revealed discrete labeling of nuclear granule structures as well as diffuse labeling in the nucleoplasm, and demonstrated co-localization of ppMVa with SC35, a splicing factor that earmarks interchromatin granule clusters in the nucleus, often referred to as “speckles”. ppMVa was not detected in other nuclear particles, such as Cajal bodies, gems, nucleoli and perinucleolar caps. Inhibition of transcription by actinomycin D induced the recruitment of ppMVa into larger interchromatin granules, as has been described for splicing factors in general. The recombinant globular tail domain of MVa, transfected into melanocytes, was endogenously phosphorylated and translocated to the nucleus. When treated with melanocyte stimulating hormone, the transfected cells took on a bipolar morphology in contrast to the multiple dendritic spread morphology of non-transfected cells. These observations identify a novel role for MVa in nuclear organization and genetic expression, offering a new lead towards the understanding of actin-based gene regulation.

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