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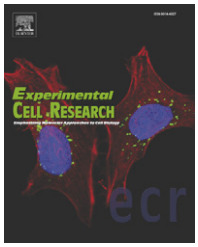

Research Article

Synemin isoforms during mouse development: Multiplicity of partners in vascular and neuronal systems

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ABSTRACT

The intermediate filament (IF) synemin gene encodes three IF proteins (H 180, M 150, L 41 kDa isoforms) with overlapping distributions. In the present study we analysed the mRNA and protein expression of each isoform in developing mouse embryos. Synemin M mRNA was present as early as E5 with vimentin and nestin. Synemin H was found later at E9 in the nervous system and mesodermic derivatives concomitantly with angiogenesis, somitogenesis and the migration of neural crest cells. Synemin L appeared later in neurons at E15. Furthermore, the synemin isoforms required different IF partners depending on the cell type to form filamentous structures. In endothelial cells, synemin H/M were found associated with vimentin and were absent in vimentin-null mice. In neurons of the peripheral nervous system of E15 embryos, synemin H/M or L were co-expressed with neurofilament, peripherin and internexin. In adult mice, our data support the existence of different subpopulations of neurons within the dorsal root ganglia: one composed of small neurons containing synemin H/M and peripherin, and another composed of large neurons containing synemin L and neurofilaments. Axons devoid of neurofilaments from mutant mice (NFHLacZ) showed an absence of the L isoform but contained H/M isoforms with peripherin.

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Introduction

In order to meet the changing needs of living cells, the cytoplasm must be dynamically modulated via viscoelastic properties that are influenced by both the protein profile of the cell and the organization of cytoplasmic components [1]. One important component is intermediate filament (IF) proteins that are encoded by a complex family of up to 70 genes [2, 3]. IF proteins are chemically heterogeneous, have different rates of assembly, and yield discrete types of filaments [4]. Their unique physical properties and interactions lead to associations with different accessory proteins and the generation of dynamic, interconnected, cell-type-specific cytoarchitectures [5]. Animal models have demonstrated that IFs are required for the cell architecture, the distribution of mitochondria and the mechanical integrity of cells [6]. Several genetic diseases are caused by perturbed IF function giving rise to fragile cells unable to sustain different physical stresses [7–9].

In spite of sequence similarities between the IF proteins, some differ in that they require other IF proteins for incorporation into polymers. In this respect, synemin [10] is similar to nestin [11, 12] and neurofilament-H (NF–H) [13] in the nervous system and syncoilin [14] in muscle. Analysis of the evolutionary history of the IF proteins fits a model in which type VI proteins form a branch distinct from neurofilament (NF) proteins, composed of two large proteins, synemin and nestin [15]. The synemin gene encodes three isoforms in mice and humans through an alternative splicing

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mechanism, giving rise to three proteins (High or alpha synemin 180 kDa, Middle or beta synemin 150 kDa or desmuslin [16], and Low synemin 41 kDa [17,18]) with the same head and rod domains but differing at the C-terminal end. Mechanisms specific to muscles or neurons probably control the splicing of the common synemin mRNA and the synthesis of each synemin isoform [19,20]. A specific exon exclusive to the α variant of synemin codes for a region which interacts directly with the focal adhesion protein vinculin in its active state [21]. Interaction between adhesion components and IFs could serve as a general mechanism regulating cell- and tissue-specific cytoskeleton–membrane attachment in muscle [22]. In astrocytoma cells with migratory properties, synemin contributes to the dynamics of the actin cytoskeleton [23]. In rat and human liver tissue, synemin acts as a bridging protein between IFs and focal adhesions [24]. Likewise the tail domain of synemin provides a binding site for desmin and alpha actinin, thus integrating IF and actin filament systems at costameres and Z-lines in muscle [10, 21]. Plectin 1 links to costameric sarcolemma through synemin, dystrobrevin and actin [25, 26]. Synemin can also act as an anchoring protein for protein kinase A (PKA) [27], the latter notably phosphorylating desmin IFs, leading to their disassembly [28]. Our previous finding of a physical association between synemin L and a membrane subpopulation of NF proteins suggests that synemin participates with other cytoskeletal proteins in the dynamic organization of the membrane compartments of neurons [19].

The results of our previous studies indicate a complex regulation of synemin gene expression and a developmental stage-specific production of each isoform. Several IF genes may therefore be activated during embryonic development [29–31]. As cells migrate during development, the IF scaffold must be able to change both rapidly and substantially. The application of tension forces have been shown to directly promote capillary outgrowth [32] as well as axon elongation in nerve cells [33]. The ability of cytoplasmic IFs to support the many modifications undergoing cells may account for their diverse primary structures and the differences in their transcriptional and post translational regulation.

This report focuses on the spatial and temporal syntheses of synemin isoforms during the development of the mouse embryo. We examined the embryonic stages during which the cells migrate from the neural crest and mesoderm to answer the following questions: First, at which stage does synemin RNAs appear and how does their presence relate to other events? Second, do the three synemin isoforms appear simultaneously? Third, what are the other partners of synemin in the peripheral nervous system? Here we have determined the temporal and spatial expression of the three synemin isoforms. By introducing the LacZ sequence into the synemin gene we analysed the tissue specific expression of synemin. Vimentin-null mice were used to demonstrate the requirement for vimentin to detect synemin. Mice with NF-deficient axons were also used to study the complexity of the associations of synemin isoforms with the different intermediate neuronal IFs in ganglia.

Materials and methods

RT-PCR analysis

Total RNA from mouse embryos aged E5 to E11 and adult mouse heart was isolated with Trizol Reagent (Invitrogen) and reverse-transcribed into cDNA with first-strand DNA synthesis kit (Roche Diagnostic) following the manufacturer's instructions. The following primers were used to detect the expression of different genes in PCR amplification as described previously [18]. For synemin H and M isoforms: SynEx4F (5′AGTCAGGGAGCGTTTCTGTGGACG) and SynEx5R (5′ATCGCTTCTCGTGTCGCTCAAATCC) located in exons 4 and 5 were used. For synemin L: SynEx2F (5′GAGAGTGATTGACA-GCCTGGAGGA) in exon 2 and SynEx5R. For vimentin: VimF (5′ GATTTCTCTGCCTCTGCCAAC) and VimR (5′GTGATGCTGAGAA-GTCTCAT); For nestin: NesF (5′AGGCTTCTCTTGGCTTTCCT) and NesR (5′TGGATCATCAGGGAAGTGGT). For syncoilin: SncF (5′ AGGAGGAGTATGAGCCTCAGC) and SncR (5′TGGAAAGCTCTTCAG-CAAGG). For GAPDH: GAPDHF (5′AGTCCATGCCATCACTGCCACCCA) and GAPDHR (5′TCCACCACCCTGTTGCTGTAGCCG). To evaluate the DNA contamination equal quantity of total RNA was used in control PCR reaction.

In situ hybridization

Two probes were prepared for synemin. The first (1.1 kb) probe which reacts with all three isoforms and spans part of exons 4 and 5 of mouse synemin, was prepared by inserting a 1085 bp fragment of the synemin gene (nucleotides 2809 through to 3894) into pBluscribe (Promega). The second (0.9 kb) that reacts only with the H isoform, corresponding to exon 4a (nucleotide 3453 through to 4359) of mouse synemin H, was amplified by PCR and ligated into pGEMT-easy (Promega) (see supplementary Fig. 1). The nestin probe, kindly supplied by Dr. Jing [34], was prepared as described [35]. In situ hybridization on Swiss mouse embryos aged from E7 to E11.5 and tissue sections was performed as described previously [36]. A digoxigenin-labelled probe, generated according to the manufacturer's protocol (Roche Diagnostics) was used.

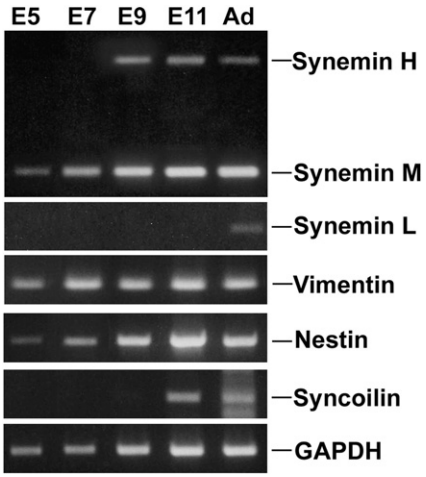

Fig. 1 – Detection of mRNAs encoding synemin, nestin, vimentin and syncoilin during embryonic development from E5 to E11 by RT-PCR. Synemin M, vimentin and nestin mRNAs were found at E5 and persisted throughout the development (E5–E11). Synemin H, L and syncoilin transcripts were absent at E5. Synemin H mRNA was first detected at E9 and syncoilin mRNA at E11 when synemin L was still absent. Adult heart RNA (Ad) was used as a positive control. The GAPDH gene was used as the reference transcript.

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