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Mechanisms of Development 117 (2002) 311–314

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Gene expression pattern

Expression profiles of the essential intermediate filament (IF) protein A2 and the IF protein C2 in the nematode *Caenorhabditis elegans*

Anton Karabinos[a], Ekkehard Schulze[b], Tiemo Klisch[a], Jian Wang[a], Klaus Weber[a,*]

[a]*Department of Biochemistry, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Goettingen, Germany*
[b]*Third Department of Zoology-Developmental Biology, University of Goettingen, Humboldtalle 34A, 37073 Goettingen, Germany*

Received 2 May 2002; received in revised form 28 May 2002; accepted 29 May 2002

Abstract

The multigene family of intermediate filament (IF) proteins in *Caenorhabditis elegans* covers 11 members of which four (A1–3, B1) are essential for development. Suppression of a fifth gene (C2) results in a dumpy phenotype. Expression patterns of three essential genes (A1, A3, B1) were already reported. To begin to analyze the two remaining RNAi phenotypes we followed the expression of the A2 and C2 proteins. Expression of A2 mRNA starts in larval stage L1 and continues in the adult. Transgenic A2 promoter/gfp larvae strongly display GFP in the main body hypodermis but not in seam cells. This pattern and the muscle displacement/paralysis induced by RNAi silencing are consistent with the role of this protein in keeping the correct hypodermis/muscle relationship during development. IF protein C2 occurs in the cytoplasm and desmosomes of intestinal cells and in pharynx desmosomes. Expression of C2 starts in the late embryo and persists in all further stages. © 2002 Published by Elsevier Science Ireland Ltd.

Keywords: *Caenorhabditis elegans*; Desmosomes; Hypodermis; Intermediate filament; Promoter

1. Results and discussion

Development of hypodermis and muscle are coordinated in *Caenorhabditis elegans* and involve interactions between extracellular matrix, cell surfaces and the cytoskeleton (Francis and Waterston, 1991; Chin-Sang and Chisholm, 2000; Hahn and Labouesse, 2001). RNAi silencing of the hypodermal intermediate filament (IF) protein A3 results in displaced body muscles and paralysis indicating an essential role of this cytoskeletal component in transmission of muscle force to the cuticle (Karabinos et al., 2001). Similar effects were observed in RNAi of the highly related protein A2 (Karabinos et al., 2001; Dodemont et al., 1994) for which expression results are lacking.

The six RNA fractions used in Northern dot blot hybridization covered the embryo, four larval stages and the adult. A2 mRNA was first detected in larval stage L1 and was abundant from the L2 stage onwards (Fig. 1A). In contrast A1 and B1 mRNAs, used as controls, were found in all developmental stages including the embryo (for other expression studies on A1 and B1, see Karabinos et al., 2001).

Comparison of the 5′-upstream sequences of the A3, and the adjacent A2 gene, identified two homology segments present in both promoters (Fig. 1B–D). The A2 promoter/gfp reporter (Fig. 1) was strongly expressed in the main body hypodermis during the entire larval development, but not in the seam cells (Fig. 2A, B). Weak expression was detected in the ventral nerve cord. These results are in good agreement with the known staining pattern of the monoclonal antibody MH4 (Francis and Waterston, 1991) which strongly stains IF proteins A1, A2 and A3 (Karabinos et al., 2001). The toxicity of A2 promoter/gfp reporter resulted in only few adults, which were significantly shorter and unhealthy. This progeny showed mosaicism for GFP expression in several tissues including vulva, uterus, cells associated with amphid sensory neurons and some unidentified neurons in the nerve cord (data not shown). Similar problems associated with GFP-expression in nematodes were reported by Eustance Kohn et al. (2000).

To analyze the dumpy C2 RNAi phenotype (Karabinos et al., 2001) we determined the developmental expression of this IF protein. Fig. 3 documents the specificity of the purified antibody against C2. Double staining with C2 and desmosomal MH27 antibodies shows that C2 is located in the cytoplasm of intestinal cells and at desmosomes in intestine and pharynx of the larva (Fig. 4A, D), late embryo and adults (data not shown). MH27 antibody additionally

* Corresponding author. Tel.: +49-551-201-1486; fax: +49-551-201-1578.
E-mail address: akarabi1@gwdg.de (K. Weber).

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PII: S0925-4773(02)00192-2

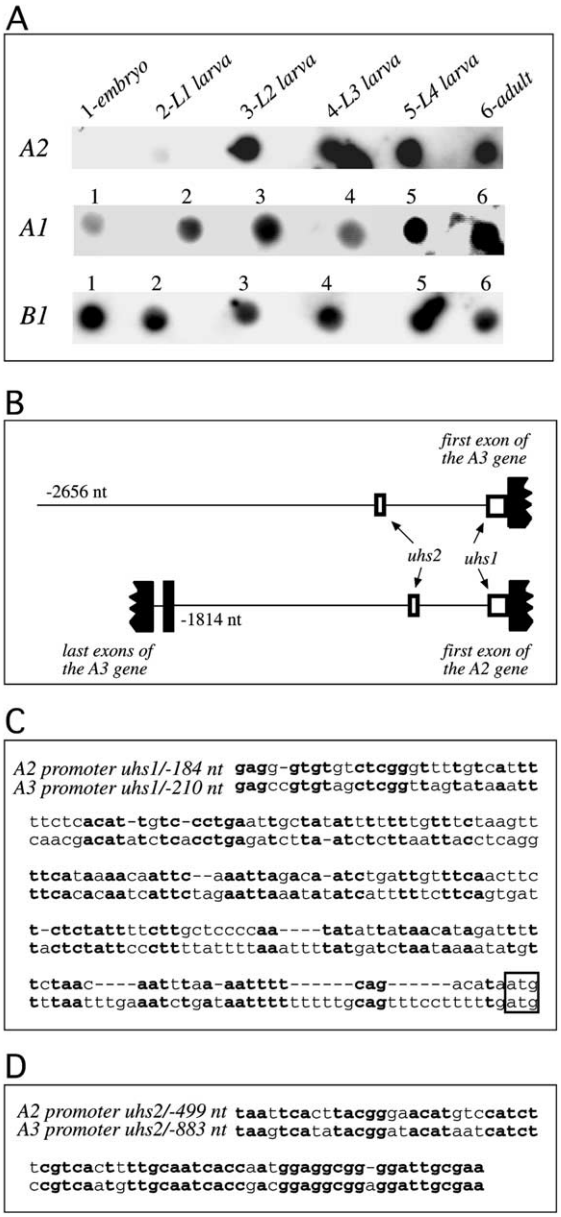

Fig. 1. (A) Northern dot blot analysis of *C. elegans* A2, A1 and B1 mRNAs during development. Each of the six dots, numbered 1–6 on the blot, contained 2 μg of total RNA from a particular developmental stage. (B) Schematic diagram of the genomic sequences including parts of the A3 and A2 genes. The upper part indicates the genomic sequence 2656 nt upstream of the A3 gene used previously in the A3 promoter/gfp construct (Karabinos et al., 2001). Below are the last two exons of the A3 gene (two dark boxes), the 1814 nt long intergenic sequence and the beginning of the A2 gene (dark box). Relative positions of two upstream homology segments uhs1 and uhs2 present in both promoter sequences are indicated by open boxes. (C) Nucleotide sequence alignment of the two upstream homology segments 1 (uhs1; 59% identity) mapping 184 and 210 nt upstream of the A2 and A3 genes, respectively. Bold letters denote identical nucleotides. Translational initiation codons ATG are boxed. (D) Nucleotide sequence alignment of the upstream homology segments 2 (uhs2; 80% identity) mapping 499 and 883 nt upstream of the A2 and A3 genes, respectively. Identity within the remaining sequences of the A2 and A3 promoters reached only about 40%. The functional importance of these two homology segments remains to be determined.

stained the hypodermal (Fig. 4B, E) and all other epithelial desmosomes (Waterson, 1988; Köppen et al., 2001).

The hypodermal co-expression of IF proteins A2 and A3 and the similarity of their RNAi phenotypes are consistent with the role of both proteins in keeping the correct spatial relationship of hypodermis and muscle cells (Karabinos et al., 2001). These results provide a framework for investigating possible interactions between the A2/A3 IF proteins and myotactin (Hresko et al., 1999), MUA-3 (Bercher et al., 2001) and MUP-4 (Hong et al., 2001) all of which have similar expression patterns. The same holds for IF protein C2 and the MH27 antigen AJM-1 which are co-expressed in pharyngeal and intestinal desmosomes. AJM-1 is an essen-

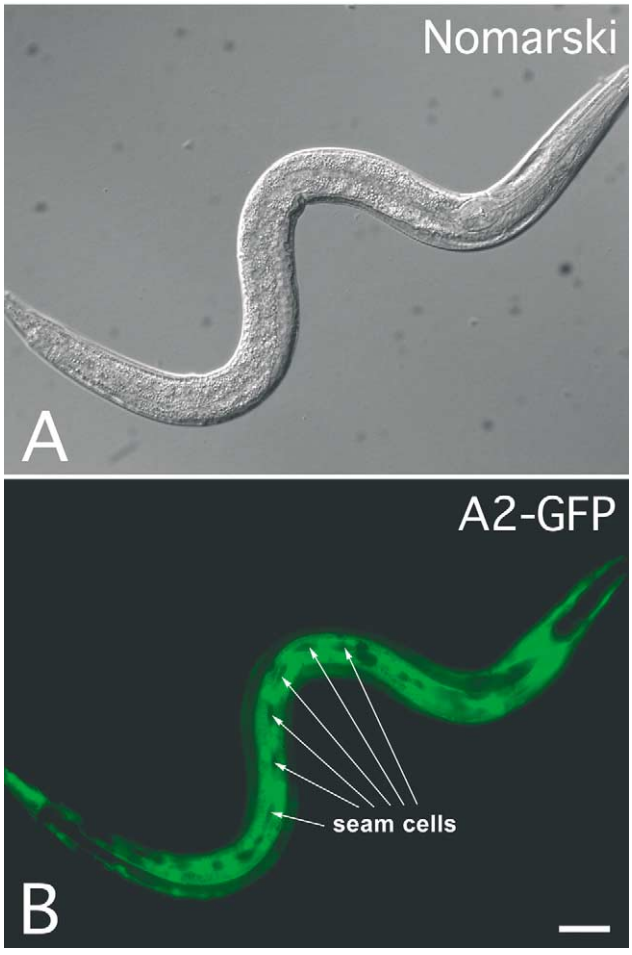

Fig. 2. Tissue specific expression of the A2-promoter/gfp construct in larva. The 1509 nt long 5′-upstream region of the A2 gene (Fig. 1B) was used in the promoter/gfp reporter. Only F2 generation animals were photographed. Due to the toxicity of the transgene it was not possible to propagate extrachromosomal arrays any further. Nomarski phase contrast monitoring (A) was used to facilitate the identification of cells and tissues. Expression of GFP in larva was detected in the main body hypodermis excluding the seam cells marked by arrows. These appear as dark gaps on the green GFP fluorescence of the hypodermal syncytium (B). Note the weak fluorescence in the ventral nerve cord and the intense GFP fluorescence of unidentified structures in the tail. Bar 25 μm.

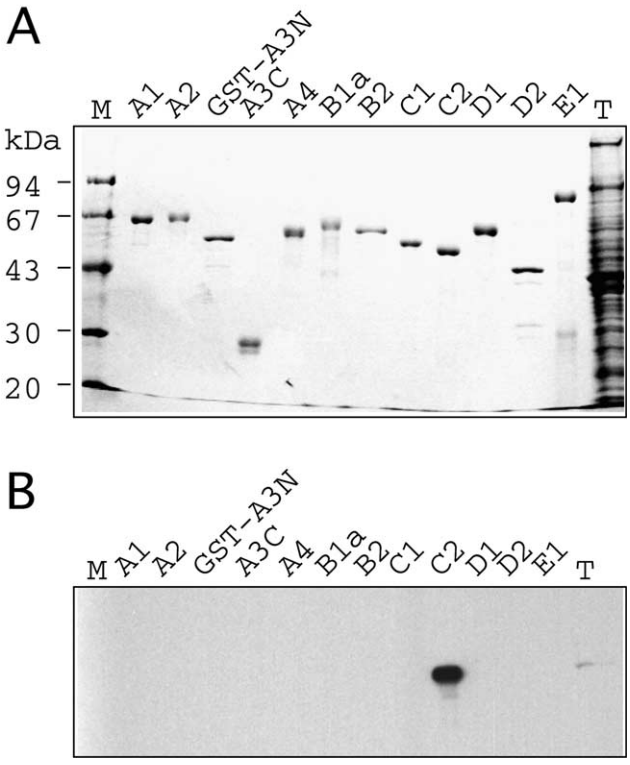

Fig. 3. Immunoblot analyses of polyclonal antibody against *C. elegans* IF protein C2. Equal amounts of recombinant IF proteins A1, A2, A4, B1a, B2, C1, C2, D1, D2, E1, the two recombinant fragments GST-A3N and A3C of A3, and a total *C. elegans* protein extract (T; see Karabinos et al., 2001) were separated by SDS-PAGE (10%) and stained with Coomassie (panel A) or blotted on to nitrocellulose membranes. Blots were incubated with affinity purified guinea pig anti-C2 antibody (panel B). The C2 antibody recognized only the recombinant C2 protein and weakly a comigrating band in the total extract. No additional band was seen after longer exposure. Marker proteins and an approximate molecular mass standard in kDa are given at the left of panel A.

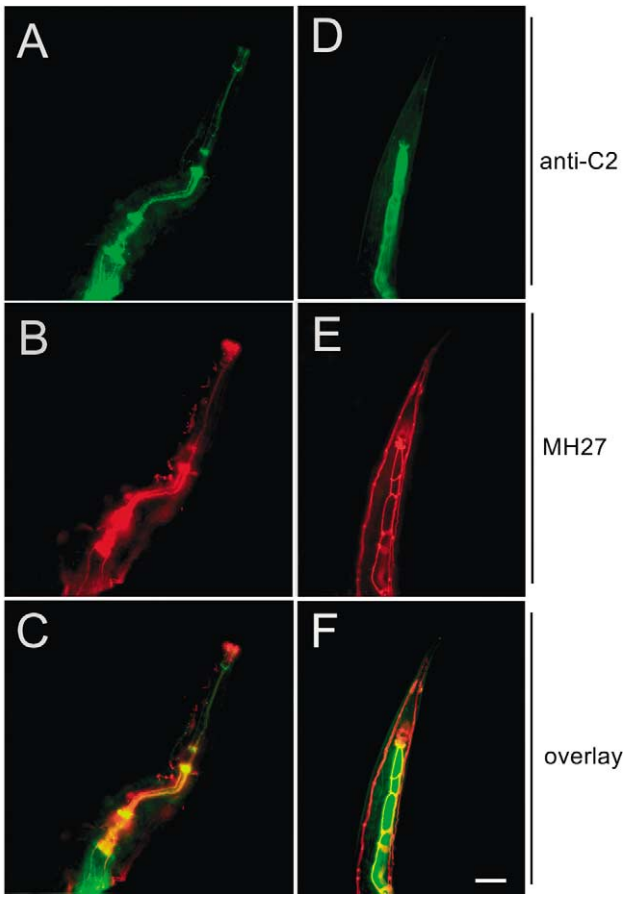

Fig. 4. Tissue specific expression of C2 protein in *C. elegans*. The double staining of whole mounts with guinea pig C2 and murine desmosomal MH27 antibodies indicates that C2 is present in the intestine and in desmosomes of pharynx and intestine (A,D) but is absent from the hypodermal desmosomes (B,E). MH27 staining of desmosomes in pharynx, intestine and hypodermis (B,E). (C,F) overlays of C2 and MH27 staining are shown in (A,B) and (D,E) respectively. Bar 25 μm

tial protein controlling junctional integrity in epithelia and contains an IF binding domain (Köppen et al., 2001).

2. Experimental procedures

General methods for *C. elegans* culture, mounting, microscopy, antibody affinity purification, immunoblotting and construction of the A2 promoter/gfp reporter were described (Karabinos et al., 2001). Extrachromosomal arrays were created by germline transformation (Mello et al., 1991). The reporter (20 ng/μl) was co-injected with pRF4 marker-plasmid (100 ng/μl; *rol-6(su1006)*; Kramer et al., 1990) into one gonad arm of strain N2 (F0 generation). Total RNAs from different developmental stages were kindly provided by Dr C. Ampe, University of Ghent. Northern blot hybridization was under high stringency conditions (Dodemont et al., 1994). RNA blots were probed with cDNA fragments described (Karabinos et al., 2001). The guinea pig anti-C2 antibody was raised using the peptide NQAKHILEQTTAHRS (Dodemont et al., 1994). Antibody dilutions: anti-C2 1:10, MH27 (Developmental Studies Hybridoma Bank, University Iowa, USA) 1:100.

Acknowledgements

This work was supported in part by the Deutsche Forschungsgemeinschaft grant SFB271 A14 to E.S.

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