



CAP2, Cyclase Associated Protein 2, is a dual compartment protein with a WH2 domain

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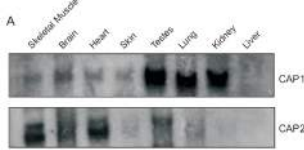
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Abstract

CAPs are evolutionary conserved proteins with roles in regulating the actin cytoskeleton and in signal transduction. Mammals have two CAP genes that code for related proteins, CAP1 and CAP2. With specific antibodies for both proteins we studied their distribution and subcellular localisation. CAP1 shows a broad tissue distribution, whereas CAP2 is significantly expressed only in brain, heart and skeletal muscle, and in skin. CAP2 is found in the nucleus in undifferentiated myoblasts and at the M-line of differentiated myotubes. In PAM212, a mouse keratinocyte cell line, CAP2 is enriched in the nucleus. By contrast, CAP1 localises to stress fibers and F-actin rich regions such as lamellipodia in PAM212 cells. In human skin CAP2 is present in all living layers of the epidermis where it localises to the nuclei and to cell-cell junctions. ChIP analysis revealed that CAP2 could effectively bind to chromatin. Moreover, like other CAPs, CAP2 can sequester actin through its C-terminal domain. This sequestering activity presumably resides in the WASP homology domain 2 (WH2 domain), a short stretch of amino acids between the N- and C-terminal domain of CAP2. Immunofluorescence studies with mouse whole mount embryos revealed the expression of CAP2 at day E12.5 specifically in the developing heart. Thus CAP2 is an important actin binding protein, which sequesters actin through its WH2 domain, with a dual compartmental distribution and may play an important role during heart development in mouse embryogenesis.

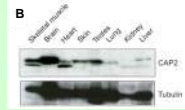
Tissue distribution of CAP2

A. CAP1 and 2 transcripts in various tissues

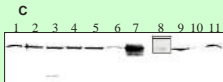


A. Northern blot analysis shows the presence of CAP1 and CAP2 transcripts in various tissues.

B & C. CAP1 and CAP2 protein in various tissues

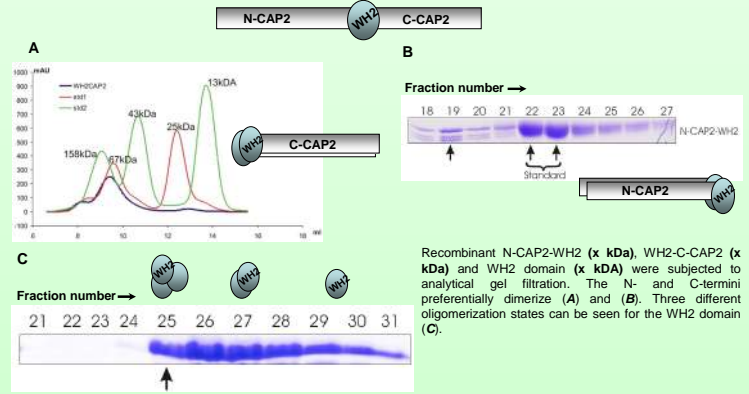


B. Western blot analysis of homogenates from various tissues shows that CAP2 is primarily present in skeletal muscle, brain, heart, skin and testis. CAP2 was recognized with polyclonal antibodies.



C. Homogenates from thymus (1), spleen (2), testis (3), lung (4), liver (5), heart (6), stomach (7), skeletal muscle (8), brain (9), skin (10), kidney (11) were probed for CAP1 with polyclonal antibodies.

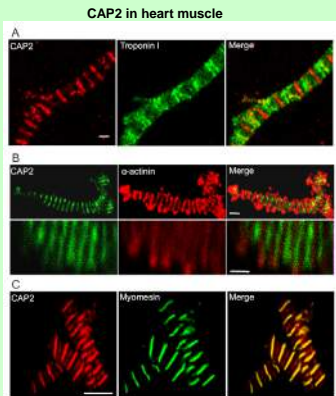
Oligomerization of different domains of CAP2



Recombinant N-CAP2-WH2 (x kDa) and WH2-C-CAP2 (x kDa) were subjected to analytical gel filtration. The N- and C-termini preferentially dimerize (A) and (B). Three different oligomerization states can be seen for the WH2 domain (C).

> CAP2 domains have the potential to oligomerize.

CAP2 localizes to the M-band in muscle



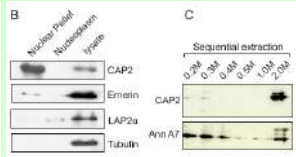
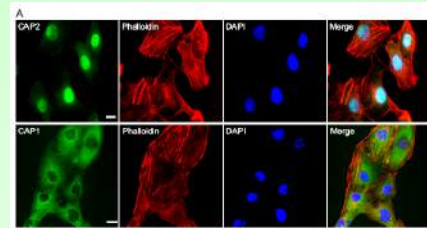
Staining with CAP2 antibodies revealed a sarcoplasmic staining pattern in the muscle cross section (A), and a striated staining pattern in the longitudinal section (B).

CAP2 is an M-band protein in heart muscle

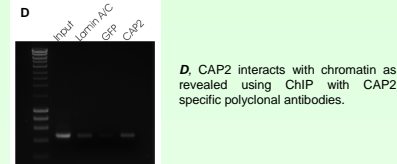
Myofibril staining with A, CAP2 specific polyclonal antibodies and cardiac specific monoclonal anti troponin I antibodies. B, CAP2 specific polyclonal antibodies and alpha-actinin specific monoclonal antibodies. C, with CAP2 and antibodies recognizing the M-band protein myomesin.

Subcellular localization of CAP in PAM212 cells

A. CAP1 and CAP2 have a different subcellular localisation in PAM212 cells.



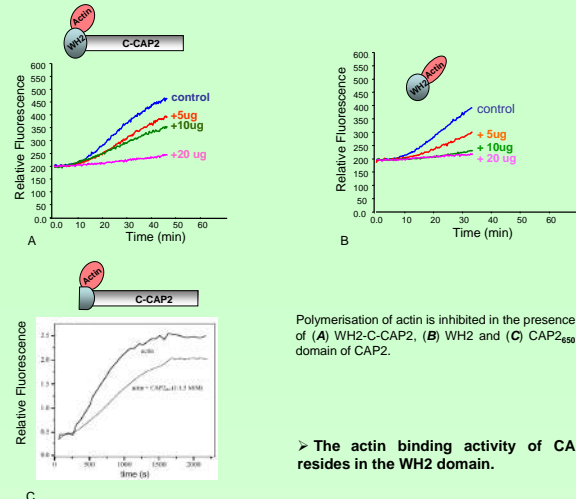
B. CAP2 stays in the nuclear pellet of PAM212 cells upon extraction using a hypotonic buffer. C. Sequential extraction of nuclei using increasing amounts of NaCl concentrations indicates a tight association of CAP2 with the nuclear matrix whereas Annexin A7 is only loosely bound.



D. CAP2 interacts with chromatin as revealed using ChIP with CAP2 specific polyclonal antibodies.

> CAP2 is a dual compartment protein
> CAP2 associates tightly with the nuclear matrix

Interaction of CAP2 with actin

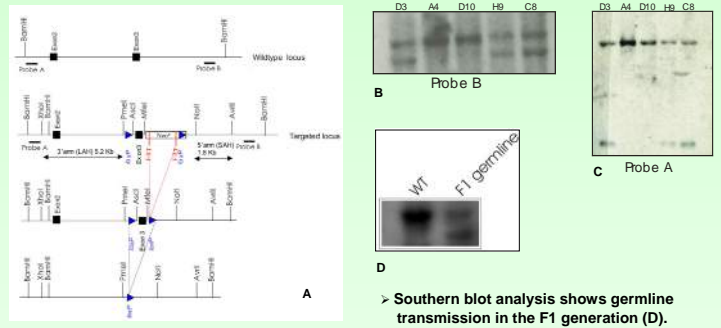


Polymerisation of actin is inhibited in the presence of (A) WH2-C-CAP2, (B) WH2 and (C) CAP2₆₅₀ domain of CAP2.

> The actin binding activity of CAP2 resides in the WH2 domain.

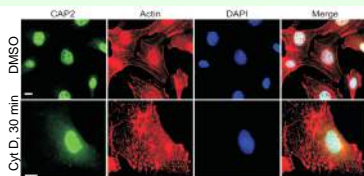
Generation of a CAP2 mouse knockout

A. Strategy for CAP2 KO generation. B and C, Southern blot analysis to verify the targeted allele in ES clones.



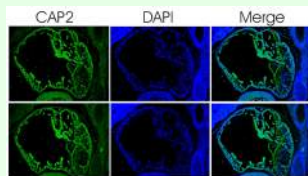
> Southern blot analysis shows germline transmission in the F1 generation (D).

Cytochalasin D treatment



CAP2 localization is not influenced by cytochalasin D treatment.

CAP2 in mouse development



In the developing embryo, CAP2 is highly expressed in heart at embryonic stage E12.5.

Summary

- > CAP2 is primarily found in brain, skeletal and cardiac muscle, and in skin.
- > CAP2 is a dual compartment protein, present in the cytosol and in the nucleus.
- > CAP2 is a component of the M-band.
- > The N- and C- domain of CAP2 can dimerize while the WH2 domain can trimerize.
- > The actin sequestering activity of CAP2 resides in the WH2 domain.