Int. J. Dev. Biol. 53: 1063-1067 (2009)
doi: 10.1387/ijdb.062158mw

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Expression of *CAP2* during early *Xenopus* embryogenesis

MARIAN WOLANSKI[#], FARHAD KHOSROWSHAHIAN[#], LARA JERANT, ING-SUAN JAP, JENNIFER BROCKMAN and MICHAEL J. CRAWFORD*

Dept. Biological Sciences, University of Windsor, Windsor, Ontario, Canada

ABSTRACT We have cloned and characterized a second member of the *Xenopus CAP (cyclase associated protein)* gene family. *xCAP2* demonstrates greater restriction of expression than its homolog, *xCAP1*, and is differentially expressed throughout early embryogenesis. Although present as a maternal transcript, *CAP2* comes to be expressed in the anterior-most mesoderm/endoderm during late gastrulation, in paraxial mesoderm during late neurula stages, and later expresses in lens, cardiac primordia, somites, otic vesicles, retina, and in the optic and craniofacial musculature. The gene is also expressed in the leading edge of myotome.

KEY WORDS: *myotome, cyclase associated protein, striated muscle, somite, cardiac, lens, retina*

Cyclase associated protein (CAP) appears to play a critical role in mediating the dynamics of actin polymerizaton in species as diverse as yeast, *Dictyostelium*, plants, fly, fish, frog, and mammal (Baum *et al.*, 2000, Benlali *et al.*, 2000, Fedor-Chaiken *et al.*, 1990, Field *et al.*, 1990, Gottwald *et al.*, 1996, Kawai *et al.*, 1998, KhosrowShahian *et al.*, 2002, Matviw *et al.*, 1992, Swiston *et al.*, 1995, Vojtek and Cooper, 1993). While the mechanisms underlying actin regulation by CAP are not yet completely understood, the protein possesses a conserved monomeric actin binding domain, as well as domains specialized for interacting with other cytoskeleton modulating proteins (Hubberstey and Mottillo, 2002). Tetrapods express two proteins, CAP1 and CAP2 which differentially express in murine non-muscle, and in striated muscle cells respectively, and in the case of CAP2, at least three transcripts are detectable by Northern blot (Bertling *et al.*, 2004). In both *Dictyostelium* and mammalian cell lines, interference with CAP results in abnormal endocytosis and cell migration, as well as abnormal cytoskeletal architecture (Bertling *et al.*, 2004, Gottwald *et al.*, 1996, Noegel *et al.*, 2004, Noegel *et al.*, 1999). Cell polarity is affected by CAP in both yeast cells and in *Drosophila* oocytes (Baum *et al.*, 2000). The developmental importance of CAP activity has been the focus of interest, and in particular the role that it plays in mediating cell change shape during morphogenetic modeling during eye development (Benlali *et al.*, 2000). It is thought that the CAP proteins compete with cofilin to bind to globular actin, and in contrast to cofilin, the CAP proteins also bind at the barbed end of filamentous actin thereby promoting depolymerization (Bertling *et al.*, 2004, Mattila *et al.*, 2004). Much of what we know about the CAPs come to us from studies of unicellular organisms or tissue culture. Relatively little is known about how the *CAP* genes express over the course of development although preliminary expression studies have been done for *CAP1* in frog (KhosrowShahian *et al.*, 2002) and to a lesser extent for *CAP2* in mice (Bertling *et al.*, 2004).

CAP2 was amplified from a *Xenopus* stage 24 cDNA library using primer sequences derived from the EST fragments available at the time (Accession number AY303832). The clone demonstrates high amino acid sequence identity with other tetrapod CAP2 proteins (Fig. 1). By Northern blot, *CAP2* is present as a maternal transcript and it exhibits a different pattern of expression relative to *CAP1* which undergoes diminution of transcriptional activity from gastrulation through to early neurulation (Fig. 2A). By contrast, *CAP2* expresses at fairly constant levels from early development through to organogenesis stages. The gene products differ in size: *CAP2* is 3.5 kb, while *CAP1* expresses as a 2.3 kb transcript. This is consistent with the sizes reported for mouse in which *CAP2* expressed as a longer transcript than *CAP1*(Bertling *et al.*, 2004). The diffuse bands evident by Northern blots for *Xenopus CAP2* may reflect multiple isoforms. Aceview for human *CAP2* reports that the gene comprises at least 17 introns and encodes at least six variant proteins. Fourteen of the intron/exon boundaries are canonical, and three are reported as "fuzzy". The transcripts vary according to 3' truncation, the vari-

Abbreviations used in this paper: CAP, cyclase associated protein.

***Address correspondence to:** Michael J. Crawford. Dept. Biological Sciences, University of Windsor, 401 Sunset Ave., Windsor, Ontario, N9B 3P4, Canada. Fax: +1-519-971-3609. e-mail: mcrawfo@uwindsor.ca

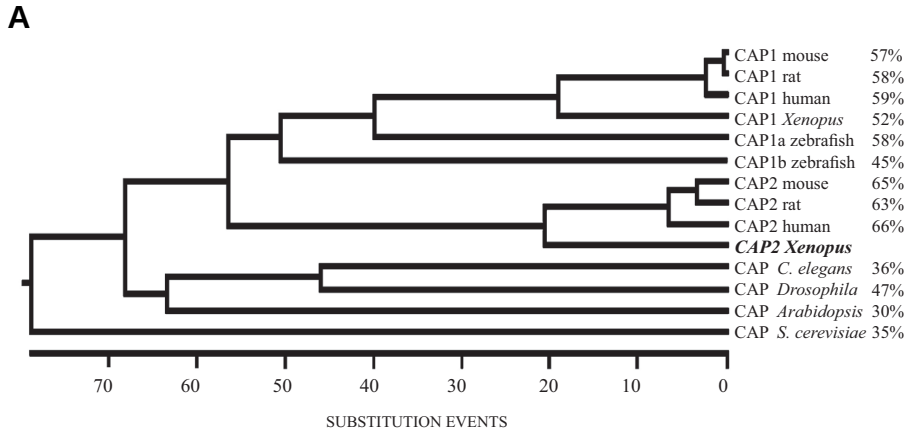
[#]Note: These authors contributed equally.

Accepted: 18 May 2008. *Published online:* 29 June 2009.

ISSN: Online 1696-3547, Print 0214-6282

able use of four exons, and different exon boundaries (Thierry-Mieg and Thierry-Mieg, 2007). Mouse *CAP2* expresses at least three isoforms (Bertling *et al*., 2004). Several different cDNAs for *CAP2* in *Xenopus tropicalis* are retrievable in Genbank, and this suggests that variable promoter usage or alternative splicing produces a diversity of *CAP2* transcripts in vertebrates generally. Semi-quantitative RT-PCR that employed primers directed to the 5' end of the gene yielded amplicons that were roughly consistent with expression patterns revealed by Northern blot analysis (Fig 2B). RT-PCR using primers to the 3' end of the gene yielded variably sized fragments that were present in differing amounts in a stage specific manner. If the *Xenopus laevis* gene has a structure close to that of *X. tropicalis*, then the 3' primer pair brackets 6 exons. One amplicon is the correct size to represent "complete" sequence, and the others are the right size to represent transcripts with one of three specific exons differentially represented (the *X. tropicalis* exons that are 83, 208, and 170 bp long). We caution that semi-quantitative RT-PCR cannot be considered appropriate for diagnosing transcript expression levels for *CAP2* since the multiplexed products do not amplify in a directly proportional manner.

In whole mount *in situ* hybridization preparations *CAP2* is first faintly detectable in the anterior-most ectoderm and endoderm at stage 14, in bleached sections, and in weakly in intact wholemounts at stage19. It becomes more prominent by stage 20 (Fig 3A). It next restricts to the paraxial mesoderm and later to the somites as they form (Fig 3B,C,D). There is expression in developing lens (stage 27-30 Fig 3D, E), as well as in cardiac primordia (Fig 3 D, E). By Contrast, *CAP1* expresses extensively in sensorial ectoderm, branchial arch, periocular mesenchyme, and lens at this stage (Fig 3D insert; KhosrowShahian *et al*., 2002), but not in cardiac primordia (Fig 3 D cardiac region white arrow). Cardiac tube expresses *CAP2* as well as facial musculature as it elaborates (Fig 3E, G), but *CAP1* is absent retina at this stage (Fig 3G insert and KhosrowShahian *et al*., 2002), Moreover, although *CAP1* is expressed in mesenchyme, it does not express in discrete muscles like *CAP2* (KhosrowShahian *et al*., 2002). Somites in *Xenopus* are a little different from those found in chicks and mammals: dermatome is present as an unsegmented sheet that sits above the somite which itself comprises largely of myotome (Newman *et al*., 1997). As the somitic myotome migrates around the flank to form the precursors of body wall muscle, *CAP2* is ex-



B

```
                  RLE (A)
Hs CAP1   MADMQNLVERLERAVGRLE----AVSHTSDMHRGYADSPSKAGAAPYVQAFDSLLAGPVAEYLKISKEIGGDVQKHA
Mm CAP1   MADMQNLVERLERAVGRLE----AVSHTSDMHCGYGDSPSK-GAVPYVQAFDSLLANPVAEYLKMSKEIGGDVQKHA
Xl CAP1   MADIHGLVDRLEKAVSKLE-SLAA---------GSGGDASSADTAVYVQAYDNLINGPVAEFMKKSKEIGGDVEKQA
Xl CAP2   MAEMDGLMDRLEKAVIRLECVLSSSYSSNSADKNVVNGINGGV-APFVEAFDALMAGTLEEYLKNSKVIGGEVEAHA
Hs CAP2   MANMQGLVERLERAVSRLE-SLSAESHRPPGNCGEVNGVIAGV-APSVEAFDKLMDSMVAEFLKNSRILAGDVETHA
Mm CAP2   MTDMAGLMERLERAVIRLE-QLSAGLDGPPRGCGEVNGVNGGV-APSVEAFDKLINSMVAEFLKNSRVLAGDVETHA
                                                  B
Hs CAP1   EMVHTGLKLERALLVTASQCQQPAENKLSDLLAPISEQIKEVITFREKNRGSKLFNHLSAVSESIQALGWVAMAPKP
Mm CAP1   EMVHTGLKLERALLATASQCQQPAGNKLSDLLAPISEQIQEVITFREKNRGSKFFNHLSAVSESIQALGWVALAAKP
Xl CAP1   DMFMSGIQLQRGILVMASQYQQPAEKDLVAILAPLSGKIQTVQEFREKNRGSKLFNHLSSLSESVPALGWVAMAPKP
Xl CAP2   GMVENAFKAERAFLVFASQHQQPPQVEESLILKPISERILEVQLFREKNRGSQMFNHLSAISESVPALGWIAMSPKP
Hs CAP2   EMVHSAFQAQRAFLLMASQYQQPHENDVAALLKPISEKIQEIQTFRERNRGSNMFNHLSAVSESIPALGWIAVSPKP
Mm CAP2   EMVHGAFQAQRAFLLMVSQYQQPQENEVAVLLKPISEKIQEIQTFRERNRGSNMFNHLSAVSESIAALGWIAVSPKP
                        C
Hs CAP1   GPYVKEMNDAAMFYTNRVLKEYKDVDKKHVDWVKAYLSIWTELQAYIKEFHTTGLAWSKTG-PVAKELSGLPS-GPS
Mm CAP1   GPFVKEMNDAAMFYTNRVLKEYRDVDKKHVDWVRAYLSIWTELQAYIKEFHTTGLAWSKTG-PVAKELSGLPS-GPS
Xl CAP1   GPYVKDMMDAAMFYTNRVLKEYKDVDKKHADWVRAYLNIWTDMQAYIKEHHTTGLTWKKTG-PKASGAAPAPA-APP
Xl CAP2   GPYVKEMTDAATFYTNRVLKDYKSTDQHHVDWVKSYLNIWSELQAYIKEYHTTGVSWGNAIYSIPPTANGGTLLQKS
Hs CAP2   GPYVKEMNDAATFYTNRVLKDYKHSDLRHVDWVKSYLNIWSELQAYIKEHHTTGLTWSKTG-PVASTVSAFSVL--S
Mm CAP2   GPYVKEMNDAATFYTNRVLKDYKHSDLRHVDWVRSYLNIWSELQAYIREHHTTGLTWSKTG-PVASTASAFSIL--S
          Poly Proline SH3                                  Verprolin D
Hs CAP1   AGSG-PPPPPPG-PPPPP--VS---TSSGSDE-SASRSALFAQINQGESITHALKHVSDDMKTHKNPALKAQSGPVR
Mm CAP1   VGSG-PPPPPPG-PPPPP--IP---TSSGSDD-SASRSALFAQINQGESITHALKHVSDDMKTHKNPALKAQSGPVR
Xl CAP1   APKACPPPPPPGGPPPPPPPAP---ASAPADD-SVSRSQLFAQLNQGENITKGLKHVSDNMKTHKNPSLKTQGGPVR
Xl CAP2   SEPSCPPPPPPPPPPPCPPPMADFEASKAKDDISQDRSALFAQLNQGETITKGLRHVSDEQKTHKNPKLRSESGPSR
Hs CAP2   SGPGLPPPPPPLPPPGPPPL---FENEGKKEESSPSRSALFAQLNQGEAITKGLRHVTDDQKTYKNPSLRAQGGQTQ
Mm CAP2   SGPGLPPPPPPPPPPGPPPP---FENEDKKEEPSPSRSALFAQLNQGEAITKGLRHVTDDKKTYKNPSLRAQG-QIR
                                                            E
Hs CAP1   SGPKPFSAPKPQT--SPSPKRATKKEPAVLELEGKKWRVENQENVSNLVIEDTELKQVAYIYKCVNTTLQIKGKINS
Mm CAP1   SGPKPFSAPKPQT--SPSPKPATKKEPALLELEGKKWRVENQENVSNLVIDDTELKQVAYIYKCVNTTLQIKGKINS
Xl CAP1   SGPKPFTSPKPTGVGAPAAKPVVKKQPPVLELEGKKWKVENQENVSNLEISDTELKQVVYIYKCVNSTLQVKGKINS
Xl CAP2   SPTKS-HTPSPTTPPAH--Q----SYPPLLELEGKKWRVEYQEGSQGLVISETELKQVVYIFKCKKTTVQVKGKINS
Hs CAP2   SPTKS-HTPSPTSPKSYPSQ----KHAPVLELEGKKWRVEYQEDRNDLVISETELKQVAYIFKCEKSTIQIKGKVNS
Mm CAP2   SPTKT-HTPSPTSPKSNSPQ----KHTPVLELEGKKWRVEYQEDRNDLVISETELKQVAYIFKCDKSTLQIKGKVNS
          E                                  F
Hs CAP1   ITVDNCKKLGLVF-DDVVGIVEIINSKDVKVQVMGKVPTISINKTDGCHAYLSKNSLDCEIVSAKSSEMNVLIPTEG
Mm CAP1   ITVDNCKKLGLVF-DDVVGIVEIINSRDVKVQVMGKVPTISINKTDGCHAYLSKNSLDCEIVSAKSSEMNVLIPTEG
Xl CAP1   ITVDNLQETWAGFLKMLVGIVEIINSKDVKVQVMGKVPTISINKTDGCHVYLSQDSLACEIISATSSEMNILVPTSG
Xl CAP2   IIIDNCAKLGLVF-DDVVGIVEVINSKNIEIQVLGKVPTISINKTGGCHVYLSENSIDCEIVSAKSSEMNILVP-HD
Hs CAP2   IIIDNCKKLGLVF-DNVVGIVEVINSQDIQIQVMGRVPTISINKTEGCHIYLSEDALDCEIVSAKSSEMNILIP-QD
Mm CAP2   ITVDNCKKFGLVF-DHVVGIVEVINSKDIQIQVMGRVPTISINKTEGCHLYLSEDALDCEIVSAKSSEMNVLVP-QD
            Actin(G)
Hs CAP1   GDFNEFPVPEQFKTLWNGQKLVTTVTEIAG.
Mm CAP1   GDFNEFPVPEQFKTLWNGQKLVTTVTEIAG.
Xl CAP1   GDFSEFPVPEQFKTVWNGQKMVTTVTEIAG.
Xl CAP2   GDYKEFPVPEQFKTSWDGSKLVTEPAEMMG.
Hs CAP2   GDYREFPIPEQFKTAWDGSKLITEPAEIMA.
Mm CAP2   DDYREFPIPEQFKTIWDGSKLVTEPAEIMA.
```

Fig. 1. Comparison and alignment of CAP amino acid sequences (A) Xenopus *CAP2 amino acid sequence has homology to other vertebrate CAP2 sequences.* **(B)** *Alignment of CAP2 putative amino acid sequence with the areas of isoform and cross species homology identified by Hubberstey and Mottillo (2002) highlighted: the RLE (or A) domain is defined as a adenylyl cyclase-interacting domain in yeast; Regions B,C, E F – homologous but with unknown function; polyproline potential SH3 protein partner interacting domain; verprolin-like actin binding motif (D); Actin-binding motif (G). Amino acids identical in all isoforms are boxed in grey. For purposes of comparative speculation* X. tropicalis *exon borders, which are similar to those for human, are indicated by inverted black triangles.*

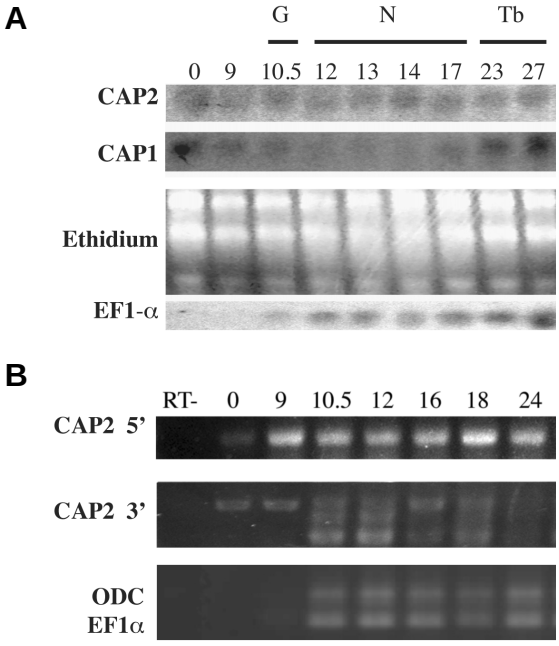


Fig. 2. Temporal expression patterns of CAP2 (A) *Northern blot analysis of* CAP2 *expression over the early stages of embryogenesis.* CAP2 *expresses in a relatively uniform pattern across stages in lanes with 10 embryo-equivalents of RNA loaded (G, gastrula; N, neurula; Tb, tailbud; Tp, tadpole), while CAP1 varies in a stage dependent manner. Few control probes express in a uniform manner across early developmental stages.* **(B)** *Semi-quantitative RT-PCR performed using primers directed to the controls elongation factor 1 α (EF1 α), and ornithine decarboxylase (ODC), as well as to 5' and 3' ends of CAP2. Stages of embryos sampled are listed across the top of the panel.*

pressed in patches on the flank that are similar to those reported for myotome patches of *Pax3, MyoD* and *Myf5* expression in *Xenopus* (Fig 3F)(Martin and Harland, 2001).

In sectioned material, *CAP2* transcript can be detected a little earlier than in wholemounts (as early as stage 14) in the anterior-most ectoderm/endoderm that is thought to play a role in patterning of head and heart (Fig.4A)(Schneider and Mercola, 1999). Sections also reveal expression in cardiac primordia (Fig. 4 B,C) as well as in the leading edge of myotome (Figs. 3F, 4D). *CAP2* expression is dynamic, differentially regulated, and commonly found in the rudiments of striated muscle (Fig. 5A) including heart, but appears to be down-regulated when the tissue is differentiated to the extent that striations are evident (compare white arrows to black arrows respectively Fig 5B). Expression in the lens (Fig 5C), is transient and the next site of eye expression is in the two plexiform layers of the retina (Fig 5 D). The outer plexiform layer is where the axons of the retinal sensory rod and cone cells impinge upon the dendrites of the bipolar cells, and the inner plexiform layer is where bipolar cell axons synapse with ganglion cell dendrites. The expression of *CAP2* in these regions is consistent with a role for the *Drosophila CAP* homolog, *act up/capulet* in regulating both eye development and the guidance and patterning of axons (Benlali *et al.*, 2000; Major and Irvine, 2005).

With the exception of the domain of anterior ectodermal/endodermal expression late in neurulation, *CAP2* predominantly expresses in cells that are undergoing cytoskeletal re-structuring in order to establish the elongated morphology necessary for the development of myotubes, lens fibers, axons and dendrites, and for somitic myotome differentiation.

Materials & Methods

Cloning and sequencing

EST sequences from *Xenopus laevis* were used to design oligonucleotides to match conserved sequences likely to represent the 5' and 3' untranslated ends of a putative *Xenopus CAP2* homolog. The oligonucle-

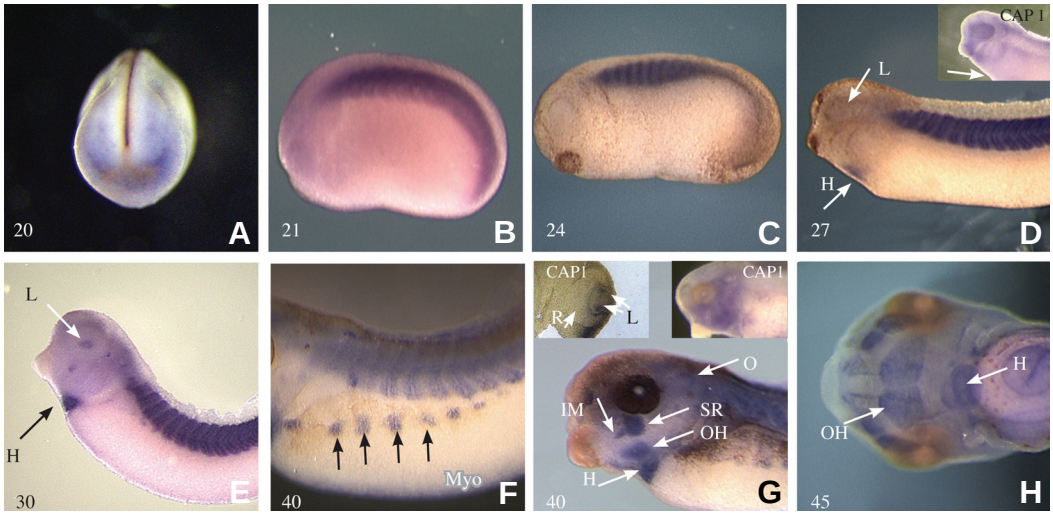


Fig. 3. Riboprobe wholemount *in situ* hybridization in *Xenopus* embryos. CAP2 *is expressed along the paraxial mesoderm and in the region where the stomadeal pocket and cement glad will form* **(A)**. *Subsequently, the paraxial mesoderm begins to segment* **(B)** *and craniofacial expression drops off as somites differentiate* **(C)**. *There is a brief period of expression in lens (white arrow) at stage 27, and in the heart (H) primordia* **(D)**. *By stage 27, heart (H) expression is well established, and remains breifly detectable in the lens (L)* **(E)**. *In contrast, at these stages CAP1 is expressed in periocular mesencyme, lens, sensorial ectoderm and branchial arch (D insert). At later stages,* CAP2 *is expressed in the precursors to striated muscle including the myotome (Myo)* **(F)**, *and in the developing heart (H), otic vesicle (O), musculus intermandibularis (IM), musculus orbitohyoidis (OH), and musculus subacuales rectis (SR)* **(G)**. *At this stage,* CAP2 *is expressed in the retina, but* CAP1 *is not, although there is an annular domain of* CAP1 *expression around the circumference of the developing lens (G left insert).* CAP1 *is expressed in branchial arches but not in retina (compare G with* CAP1 *right insert). Expression seems to diminish when the muscles differentiate to the point of displaying visible striations (compare OH and Heart in G vs. H). Embryonic stages are indicated in the lower left corner of each photograph, and bars with numbers represent scale in micrometers.*

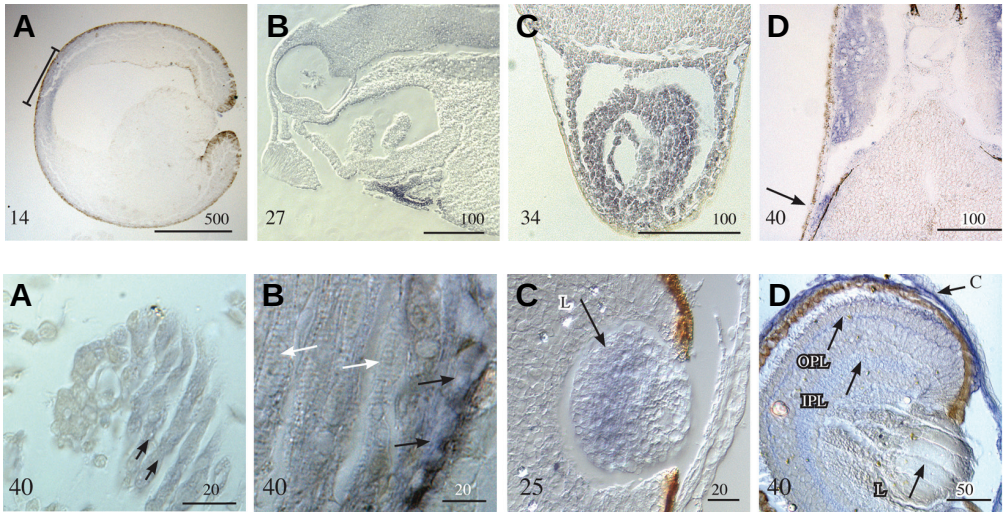


Fig. 4 (Top). Expression of *CAP2* in sectioned embryos. *The anterior end of the embryo displays staining in the region that ultimately generates the cement gland (bracket)* **(A)**. *At later stages, expression is up-regulated in presumptive heart* **(B)**, *and expresses predominantly in the mesodermally derived tissues of this organ when it forms a tube* **(C)**. CAP2 *expressing myotome migrates ventrally and around the flank from the somites* **(D)**.

Fig. 5 (Bottom). Expression of CAP2 in sectioned muscle and eye. CAP2 *is also expressed in the myoblasts as they aggregate and fuse into tubes (arrows)* **(A)**, *but transcription begins to diminish when the muscle fibers have differentiated sufficiently to show striations (compare relatively undifferentiated dark arrow with striated white arrows* **(B)**. *Expression in lens (L) is evident in the domain of forming lens fibers (see arrow in* **(C)**), *but it disappears rapidly thereafter whereupon the outer and inner plexiform layers (OPL and IPL, respectively) of the retina, as well as the chorion (C) transcribe the gene* **(D)**.

otides were designed to provide an *XbaI* and *BamHI* site at the 5' and 3' ends respectively. (*XbaI* 5' end – GCT CTA GAG ATG CCT ATC TAG CTA GG; *BamHI* 3' end – TCT GGA TCC TGT GTG GAA TAC TAA ATG G). This produced a 1947 bp amplicon that was bi-directionally sequenced using dideoxy chain termination chemistry, and the open reading frame of which was subcloned into pCS2 using *EcoRI* and *XbaI* adapted oligonucleotides (5' end - GTG AAT TCG AGC AGG CAG GTA TTC AAA ATG GC; 3' end – GCT CTA GAC TAT CCC ATC ATT TCT GCA GG)(Accession number AY303832).

Northern blot analysis

Embryos of each stage of interest were homogenized in Trizol (Invitrogen Inc) and extracted for total RNA. Ten embryo equivalents were loaded into each lane of a formaldehyde agarose gel, and the Northern blot performed and probed essentially as previously described (Crawford *et al*., 2001), but using random primed probes specific for *Elongation factor 1 alpha, CAP1*, and *CAP2*.

Semi-quantitative RT-PCR

Purifications of RNA from each of the stages were accomplished by isolation through Trizol (Invitrogen). From each of the sampled stages, mRNA equivalent to one embryo was withdrawn and cDNA synthesized in the presence on RNasin (Promega) using reverse transcriptase according to the manufacturer's instructions (Omniscript, Qiagen). One fifth volume of this reaction was employed as template for amplification. PCR conditions were determined empirically to establish the linear range of amplification for *CAP2.* Reactions were accomplished using Taq polymerase (MBI Fermentas) in 10mM Tris (pH 9.0), 50mM KCl, 0.1% Triton X-100, 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.1mM [^{32}P]dCTP, and 1 uM of each primer. Control primers were: *EF1-α* – CAGATTGGTGCTGGATATG and ACTGCCTTGATGACTCCTA; ODC – GTCAATGATGGAGTGTATG and TCCATTCCGCTCTCCTGA. Initial denaturation was for 3 minutes at 94ºC, and cycling parameters were repeated 29 times at 94ºC for 45 seconds, 57ºC for 1 minute, and 74ºC for 45 seconds. One tenth of each reaction was run out on 2% agarose in 1X TAE. Two different primer sets were employed to assess CAP2 expression levels: a 5'-specific pair that produced an amplicon comprising 496 base pairs (5'-GATGTGCAGGAGGTGTAGT-3' and 5'-GAGATGACTGATGCTGCTA-3'), and a 3'-specific set which amplified a 508 base pairs fragment (5'-TCCTGCACATCAGTCTTAT-3' and 5'-AAAGCACTGACCAACACCATGT-3'). Initial denaturation was for 2 minutes at 94ºC, and cycling parameters were repeated 31 times at 94ºC for 45 seconds, 55ºC for 45 seconds, and 74ºC for 30 seconds.

Phylogenetic comparisons

Amino acid sequences for various animal CAP proteins were clustered using CLUSTALW (Higgins and Sharp, 1989). Sequences employed for CAP1 were: *S. cerevisiae* – A34896; *A. thaliana* – NP195175.1; *C. elegans* – NP510713.1; *D. melanogaster* – NP524806; *D. rerio* - NP510713.1 and AY162326; *X. laevis* – AF411959; *M. musculus* – I49572; *R. norvegicus* – A46584; *H. sapiens* – Q01518. Sequences employed for CAP2 were: *X. laevis* – AY303832; *M. musculus* - NP080332; *H. sapiens* - NP006357.1; *R. norvegicus* - NP446326.1.

Wholemount riboprobe in situ *hybridization*

Embryos were reared according to university, provincial and federal regulations and then staged (Nieuwkoop and Faber, 1967). Wholemount *in situ* hybridizations were performed according to Harland (1991). Digoxygenein labeled sense and antisense riboprobes for *CAP2* were generated from full-length linearized template. *In situ* hybridizations were thrice repeated in embryos derived from different egg clutches, and using three different batches of riboprobe. Sections were prepared from wholemount material by embedding in paraffin wax and sectioning at 10 um.

Acknowledgements

Support for this work was provided by the Natural Sciences and Engineering Research Council of Canada in the form of grants to M.C.(203549) and by the E.A. Baker Foundation. F.KS. and M.W. were supported by NSERC postgraduate or Ontario Graduate Scholarship and Awards.

References

BAUM, B., LI, W. and PERRIMON, N. (2000). A cyclase-associated protein regulates actin and cell polarity during Drosophila oogenesis and in yeast. *Curr Biol* 10: 964-973.

BENLALI, A., DRASKOVIC, I., HAZELETT, D.J. and TREISMAN, J.E. (2000). act up controls actin polymerization to alter cell shape and restrict Hedgehog signaling in the Drosophila eye disc. *Cell* 101: 271-281.

BERTLING, E., HOTULAINEN, P., MATTILA, P.K., MATILAINEN, T., SALMINEN, M. and LAPPALAINEN, P. (2004). Cyclase-associated protein 1 (CAP1) promotes cofilin-induced actin dynamics in mammalian nonmuscle cells. *Mol Biol*

Cell 15: 2324-2334.

CRAWFORD, M.J., KHOSROWSHAHIAN, F., LIVERSAGE, R.A. and VARMUZA, S.L. (2001). *Xenopus* adenine nucleotide translocase mRNA exhibits specific and dynamic patterns of expression during development. *Biochem Cell Biol* 79: 113-121.

FEDOR-CHAIKEN, M., DESCHENES, R.J. and BROACH, J.R. (1990). SRV2, a gene required for RAS activation of adenylate cyclase in yeast. *Cell* 61: 329-340.

FIELD, J., VOJTEK, A., BALLESTER, R., BOLGER, G., COLICELLI, J., FERGUSON, K., GERST, J., KATAOKA, T., MICHAELI, T., POWERS, S. *et al.* (1990). Cloning and characterization of CAP, the S. cerevisiae gene encoding the 70 kd adenylyl cyclase-associated protein. *Cell* 61: 319-327.

GOTTWALD, U., BROKAMP, R., KARAKESISOGLOU, I., SCHLEICHER, M. and NOEGEL, A.A. (1996). Identification of a cyclase-associated protein (CAP) homologue in Dictyostelium discoideum and characterization of its interaction with actin. *Mol Biol Cell* 7: 261-272.

HIGGINS, D.G. and SHARP, P.M. (1989). Fast and sensitive multiple sequence alignments on a microcomputer. *Comput Appl Biosci* 5: 151-153.

HUBBERSTEY, A.V. and MOTTILLO, E.P. (2002). Cyclase-associated proteins: CAPacity for linking signal transduction and actin polymerization. *Faseb J* 16: 487-499.

KAWAI, M., AOTSUKA, S. and UCHIMIYA, H. (1998). Isolation of a cotton CAP gene: a homologue of adenylyl cyclase- associated protein highly expressed during fiber elongation. *Plant Cell Physiol* 39: 1380-1383.

KHOSROWSHAHIAN, F., HUBBERSTEY, A.V. and CRAWFORD, M.J. (2002). CAP1 expression is developmentally regulated in *Xenopus*. *Mech Dev* 113: 211-214.

MAJOR, R.J. and IRVINE, K.D. (2005). Influence of Notch on dorsoventral compartmentalization and actin organization in the Drosophila wing. *Development* 132: 3823-3833.

MARTIN, B.L. and HARLAND, R.M. (2001). Hypaxial muscle migration during primary myogenesis in *Xenopus laevis*. *Dev Biol* 239: 270-280.

MATTILA, P.K., QUINTERO-MONZON, O., KUGLER, J., MOSELEY, J.B., ALMO, S.C., LAPPALAINEN, P. and GOODE, B.L. (2004). A high-affinity interaction with ADP-actin monomers underlies the mechanism and *in vivo* function of Srv2/cyclase-associated protein. *Mol Biol Cell* 15: 5158-5171.

MATVIW, H., YU, G. and YOUNG, D. (1992). Identification of a human cDNA encoding a protein that is structurally and functionally related to the yeast adenylyl cyclase-associated CAP proteins. *Mol Cell Biol* 12: 5033-5040.

NEWMAN, C.S., GROW, M.W., CLEAVER, O., CHIA, F. and KRIEG, P. (1997). Xbap, a vertebrate gene related to bagpipe, is expressed in developing craniofacial structures and in anterior gut muscle. *Dev Biol* 181: 223-233.

NOEGEL, A.A., BLAU-WASSER, R., SULTANA, H., MULLER, R., ISRAEL, L., SCHLEICHER, M., PATEL, H. and WEIJER, C.J. (2004). The cyclase-associated protein CAP as regulator of cell polarity and cAMP signaling in Dictyostelium. *Mol Biol Cell* 15: 934-945.

NOEGEL, A.A., RIVERO, F., ALBRECHT, R., JANSSEN, K.P., KOHLER, J., PARENT, C.A. and SCHLEICHER, M. (1999). Assessing the role of the ASP56/CAP homologue of Dictyostelium discoideum and the requirements for subcellular localization. *J Cell Sci* 112 (Pt 19): 3195-3203.

SCHNEIDER, V.A. and MERCOLA, M. (1999). Spatially distinct head and heart inducers within the *Xenopus* organizer region. *Curr Biol* 9: 800-809.

SWISTON, J., HUBBERSTEY, A., YU, G. and YOUNG, D. (1995). Differential expression of CAP and CAP2 in adult rat tissues. *Gene* 165: 273-277.

THIERRY-MIEG, D. and THIERRY-MIEG, J. (2006). AceView: a comprehensive cDNA-supported gene and transcripts annotation. *Genome Biol.* 7 Suppl 1: S12.1-14.

VOJTEK, A.B. and COOPER, J.A. (1993). Identification and characterization of a cDNA encoding mouse CAP: a homolog of the yeast adenylyl cyclase associated protein. *J Cell Sci* 105: 777-85.

Further Related Reading, published previously in the *Int. J. Dev. Biol.*

See our recent Special Issue ***Fertilization***, in honor of David L. Garbers and edited by Paul M. Wassarman and Victor D. Vacquier at:
http://www.ijdb.ehu.es/web/contents.php?vol=52&issue=5-6

Expression of Bmp ligands and receptors in the developing Xenopus retina
Jennifer C. Hocking and Sarah McFarlane
Int. J. Dev. Biol. (2007) 51: 161-165

Myoskeletin, a factor related to Myocardin, is expressed in somites and required for hypaxial muscle formation in Xenopus
Hui Zhao, Martha L. Rebbert and Igor B. Dawid
Int. J. Dev. Biol. (2007) 51: 315-320

The Xenopus ortholog of the nuclear hormone receptor Nr2e3 is primarily expressed in developing photoreceptors.
Martinez-De Luna, R.I. and El-Hodiri, H.M.
Int. J. Dev. Biol. (2007) 51, 235-40.

Retinoic acid is required for specification of the ventral eye field and for Rathke's pouch in the avian embryo.
Maden, M., Blentic, A., Reijntjes, S., Seguin, S., Gale, E. and Graham, A.
Int. J. Dev. Biol. (2007) 51, 191-200.

Temporal and spatial expression patterns of FoxN genes in Xenopus laevis embryos.
Schuff, M., Rossner, A., Donow, C. and Knochel, W.
Int. J. Dev. Biol. (2006) 50, 429-34.

Evolving eyes
Russell D. Fernald
Int. J. Dev. Biol. (2004) 48: 701-705

From embryonic induction to cell lineages: revisiting old problems for modern study
Tokindo S. Okada
Int. J. Dev. Biol. (2004) 48: 739-742

Conservation and non-conservation of genetic pathways in eye specification
Amy L. Donner and Richard L. Maas
Int. J. Dev. Biol. (2004) 48: 743-753

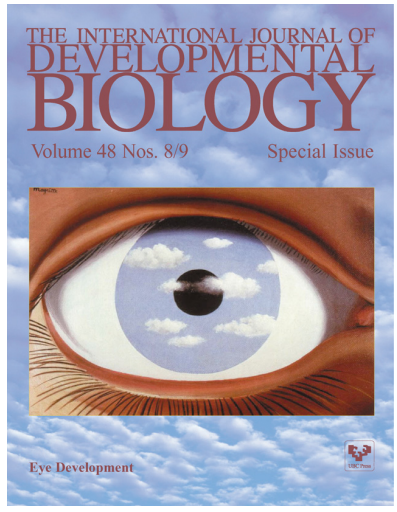
Regulation of vertebrate eye development by Rx genes
Travis J. Bailey, Heithem El-Hodiri, Li Zhang, Rina Shah, Peter H. Mathers and Milan Jamrich
Int. J. Dev. Biol. (2004) 48: 761-770

A re-examination of lens induction in chicken embryos: in vitro studies of early tissue interactions
Charles H. Sullivan, Leslie Braunstein, Royce M. Hazard-Leonards, Anna L. Holen1, Fouad Samaha, Laurie Stephens and Robert M. Grainger
Int. J. Dev. Biol. (2004) 48: 771-782

Pathways regulating lens induction in the mouse
Richard A. Lang
Int. J. Dev. Biol. (2004) 48: 783-791

Analysis of mouse eye development with chimeras and mosaics
J. Martin Collinson, Robert E. Hill and John D. West
Int. J. Dev. Biol. (2004) 48: 793-804

Regulation of gene expression by Pax6 in ocular cells: a case of tissue-preferred expression of crystallins in lens
Ales Cvekl, Ying Yang, Bharesh K. Chauhan and Kveta Cveklova
Int. J. Dev. Biol. (2004) 48: 829-844



5 yr ISI Impact Factor (2008) = 3.271