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A cDNA library of the eutardigrade *Hypsibius klebelsbergi* Mihelčič, 1959 and analysis of the actin gene

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ABSTRACT

A cDNA library was constructed from the glacier-dwelling eutardigrade Hypsibius klebelsbergi *from more than 2000 individuals collected in the Austrian Central Alps. RNA, DNA and proteins were successively isolated by the Trizol®-method. From the RNA preparation a cDNA library was constructed with the cDNA inserted unidirectionally in the phagemid expression vector TriplEx2. The primary gene library had a titre of 10^7 pfu ml^{-1} and the final amplified gene library a titre of 6×10^9 pfu ml^{-1}. The average insert length was about 1.6 kb. The partial sequence of* H. klebelsbergi *actin (746 bp) showed highest similarity to GenBank data of* Drosophila melanogaster *actin at the nucleic acid level (84.9%) and at the amino acid level (98%). Compared with actin fragments of the eutardigrades* Ramazzottius oberhaeuseri *(450 bp) and* Macrobiotus *sp. (453 bp) the identities were 85% - 81% and 100% - 98% with respect to the nucleic/amino acids. Identity with actin fragments (359 bp) of* Hypsibius dujardini *from GenBank was 96% - 100%.*

Key words: Eutardigrada, cDNA library, mRNA, actin

1. INTRODUCTION

Since 1996 a variety of molecular investigations using 18S rDNA have been published to elucidate phylogenetic and intergeneric relationships of Tardigrada (e.g. Garey *et al*. 1996, 1999; Giribet *et al*. 1996; Moon & Kim 1996; Aguinaldo *et al*. 1997; Garey 2001; Jørgensen & Kristensen 2004; Nichols *et al*. 2006; Kiehl *et al*. 2007, this issue). Only a few data exist on proteins derived from nucleic acid sequences (Regier *et al*. 2004; Schill *et al*. 2004).

A useful prerequisite for a detailed analysis of proteins is the construction of a cDNA library that allows the production of recombinant proteins. With respect to tardigrades only a single cDNAgene- library exists from the eutardigrade *Hypsibius dujardini* (Doyère, 1840) (TardiBase, Daub *et al*. 2003). The authors bred this species to obtain a sufficient number of specimens for RNA isolation and construction of an Expressed Sequence Tag (EST) library. From this library a considerable number of sequenced fragments of mRNAs have been incorporated in GenBank. For detailed protein analysis a cDNA expression library is necessary. However, tardigrades used for this purpose should be available in numbers of more than a thousand, they should be correctly identified to exclude species-specific protein variations, and contamination with foreign nucleic acids should be reduced to a minimum.

To overcome these difficulties we used the glacier dwelling eutardigrade *Hypsibius klebelsbergi* Mihelčič, 1959. This unique species lives in great numbers in cryoconite holes of several glaciers in the Alps. It can easily be collected and survives a long period at 2-4 °C when kept in water from the cryoconite holes supplemented occasionally by distilled water (Dastych *et al*. 2003). Due to the dark brown coloration of adults, *H. klebelsbergi* can be easily distinguished from other eutardigrades occasionally present in the same habitat (Dastych *et al*. 2003).

We report here on the construction of a cDNA library of *H. klebelsbergi* and on the sequence analysis of one actin isoform. Actin is one of the most highly conserved proteins and only its end terminal portions show some variability in isoforms. The actin filaments, part of the cytoskeleton of all eucaryotic cells, are formed by polymerization of monomer globular actin in muscles and non-muscle cells consisting of 374 - 376 amino acids. The polymer state of actin is regulated by a great variety of actin binding proteins that probably vary among taxa (Kabsch & Vandekerckhove 1992; Vandekerckhove 1993). The first results presented herein are part of a wider study that aims to characterise tardigrade actin and its isoforms and different actin binding proteins. Such findings will give new insights in functional adaptations and should enrich the discussion about the phylogenetic position of tardigrades.

2. METHODS

Hypsibius klebelsbergi was collected from cryoconite holes on the glacier Langtalferner, 2580 m a.s.l., in the Ötztal Alps (Austria). The animals were kept at approximately 4 °C until use. The water from the collecting site was occasionally complemented by frozen distilled water.

Tab. 1. Data of the constructed cDNA library of *Hypsibius klebelsbergi*. pfu = plaque forming unit.

1 - Titer of the primary gene library (volume: 500 µl)	10^7 pfu ml^{-1}
2 - Titer of amplified gene library (volume: 11 ml)	6×10^9 pfu ml^{-1}
3 - Clones with insert	>99%
4 - Insert length	~ 1.6 kb
5 - Vector	phagemid λ TriplEx2 (Clontech)
6 - Selectable marker	Ampicillin resistance
7 - Host cells	*E. coli* XL1 blue
8 - Cloning site	SfiIA, SfiIB

For construction of the cDNA library we used approximately 2000 animals. RNA, DNA and protein were successively isolated according to Chomczynski & Sacchi (1987) using the commercial phenol mixture Trizol® (GibcoBRL, Karlsruhe, Germany). In case of a limited sample size Trizol extraction allows a subsequent construction of a genomic library and the analysis of proteins. An extra reprecipitation of the RNA at the end of the procedure removed any remaining phenol traces. RNA and DNA were quantified spectrophotometrically (Beaven *et al*. 1955; Holtzhauer 1995). The protein-amount was calculated according to Warburg & Christian (1941; see also Holtzhauer 1995).

Synthesis of complete cDNA (including complete 5' ends) and cDNA library construction were carried out following the instructions recommended by the manufacturers (BD Biosciences SMART cDNA library construction Kit, Clonetech, Palo Alto, USA, Gigapack® III Gold Packaging Extract, Stratagene, La Jolla, USA). According to the manufacturer, the resulting phage lambda gene library should contain the cDNA inserted into the expression vector λTriplEx2 in the correct orientation for expression.

Actin fragments were amplified by using the primers Act U235 (AAYTGGGAYGAYATGGARAA) and Act L667 (GCCATYTCYTGYTCRAARTC) (Fretz & Spindler 1999) and the general actin primers Actup (ATG GTN GGN ATG GGN CAR AAR) and Actdown (ATG GTN GGN ATG GGN CAR AAR) (Viray Master, Chicago, 1993). PCR was run with 15 min initial denaturation at 94 °C followed by 35 cycles of 30 sec, denaturation at 94 °C, 1 min annealing at 50 °C, and 3 min elongation at 72 °C. The program ended with 20 min incubation at 72 °C. The sample volume was 25 µl. We used sample concentrations of 0.5 mM each dNTP, 0.5 µM each primer, 6m M Mg^{++}, 1.25 U Taq (Biomaster, Windeck, Germany) and 1 µl cDNA gene library as template DNA in 1× PCR buffer (Biomaster, Windeck, Germany). PCR-products were separated in 1.2% agarose gels in TBE (90 mM Tris, 90 mM boric acid, 2 mM EDTA, 1 µg ml^{-1} ethidiumbromide) and photographed under UV-light. PCR-products were sequenced by Seqlab (Göttingen, Germany).

Sequences of *H. klebelsbergi* (Accession number AM 501548) were aligned with "Align plus 2.0" (Scientific & Educational Software, Durham, USA), and compared with actin fragments obtained from *R. oberhaeuseri* (Accession number AM 501547), *Macrobiotus* sp. (Accession number AM 501546) (see Kaldeweier 2005), *H. dujardini* (Accession number CK 326228), *Drosophila melanogaster* (Meigen, 1930) actin5c (Accession number NCBI AE003435) (Adams *et al*. 2000).

3. RESULTS AND DISCUSSION

From about 2000 tardigrades carefully sorted out from the collection, we purified 17 µg of RNA, 8.4 µg of DNA and 302 µg of protein. The construction of the cDNA gene library started with 1 µg total RNA and resulted in a primary gene library with a titre of 107 pfu ml^{-1} and a volume of 500 µl. The average insert length was about 1.6 kb. The primary gene library was amplified starting with 5×10^5 potentially different clones. The titre of the final gene library was 6×10^9 pfu ml^{-1} in a total of 11 ml. Non-recombinant clones were below 1% (Tab. 1).

The titres of our primary and amplified gene library suggest that they consist of sufficient independent clones to represent most of the expressed genes of the animals. As only black-brown, i.e. more or less adult tardigrades were used, protein messages differentially expressed, for instance in various developmental stages or under special environmental conditions (e.g. freezing below or above habitat temperature) are absent.

We amplified parts of the actin sequence using the cDNA gene library as template for PCR. The various PCR products obtained by the combination of different primer pairs were sequenced and the sequences were combined. This resulted in a 746 bp long fragment of a *H. klebelsbergi* actin. Apparently this fragment represents the actin isoform, which is predominantly amplified under the experimental conditions used.

This sequence showed an 84.9% identity compared to the actin isoform ("Drome Act5c") of *Drosophila melanogaster* on the nucleic acid level and 98% identity at the amino acid level. In the case of the five different amino acids, three of the hydrophobic amino acids were conservatively exchanged; alanin was exchanged by serine in two cases (Fig. 1). Comparing corresponding regions of the actin sequences of the eutardigrades *R. oberhaeuseri* (450 bp) and *Macrobiotus* sp. (453 bp), an 85% and 81% identity was detected at the gene level and a 100% and 98% identity at the amino acid level. Values for the eutardigrade *H. dujardini* (359 bp from the GenBank) were 96% and 100%.

a

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                                   ATGGTNGGNATGGGNCARAA
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Drome Act5c    ATGTGTGACGAAGAAGTTGCTGCTCTGGTTGTCGACAACGGCTCTGGCATGTGCAAGGCC
Hypdu actin    ATG---GAAGACGAAGTTGCCGCCTTGGTCGTGGACAATGGATCCGGTATGTGCAAGGCC
Hypkl actin    ------------------------------------------------------------
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------

Drome Act5c    GGATTTGCCGGAGACGATGCTCCCCGCGCCGTCTTCCCATCGATTGTGGGACGTCCCCGT
Hypdu actin    GGATTTGCCGGAGATGACGCTCCCCGCGCCGTCTTCCCCTCCATCGTTGGCCGACCCCGT
Hypkl actin    ------------------------------------------------------------
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------

                                   ATGGTNGGNATGGGNCARAA
Drome Act5c    CACCAGGGTGTGATGGTCGGCATGGGCCAGAAGGACTCGTACGTGGGTGATGAGGCGCAG
Hypdu actin    CATCAGGGTGTCATGGTCGGTATGGGTCAAAAGGACAGCTACGTCGGTGATGAGGCCCAG
Hypkl actin    ------------------------------------------------------------
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------

                                                                     AAYTGG
Drome Act5c    AGCAAGCGTGGTATCCTCACCCTGAAGTACCCCATTGAGCACGGTATCGTGACCAACTGG
Hypdu actin    AGCAAGCGCGGTATCCTGACGCTCAAGTACCCCATCGAGCACGGCATCGTCACCAACTGG
Hypkl actin    ------------------------------------------------------------
Ramob actin    -----------------------------------------------------TAACTGG
Macsp actin    ------------------------------------------------------AATTGG

               GAYGAYATGGARAA
Drome Act5c    GACGATATGGAGAAGATCTGGCACCACACCTTCTACAATGAGCTGCGTGTGGCACCCGAG
Hypdu actin    GATGACATGGAGAAGATCTGGCATCACACCTTCTACAACGAGCTCCGCGTGGCTCCCGAG
Hypkl actin    ---------GAGAAGATCTGGCATCATACCTTCTACAACGAGCTCCGCGTGGCACCCGAG
Ramob actin    GATGATATGGAAAAGATCTGGCATCATACCTTCTACAATGAACTCCGTGTTGCCCCTGAG
Macsp actin    GATGATATGGAAAAGATCTGGCACCACACCTTCTACAACGAGCTCCGCGTGGCCCCCGAG
                        ** *********** ** *********** ** ** ** ** ** ** ***

Drome Act5c    GAGCACCCCGTGCTGCTGACCGAGGCCCCGCTGAACCCCAAGGCCAACCGTGAGAAGATG
Hypdu actin    GAACACCCCGTCCTCCTGACTGAGGCTCCCCTCAACCCCAAGGCCAACAGGGAAAAGATG
Hypkl actin    GAGCACCCCGTCCTCCTGACTGAGGCTCCCCTCAACCCCAAGGCCAACAGGGAAAAGATG
Ramob actin    GAACACCCCGTCCTCCTCACTGAGGCCCCGCTCAACCCCAAGGCCAACAGAGAAAAGATG
Macsp actin    GAGCACCCCGTCCTGCTCACTGAGGCCCCCCTGAACCCCAAGGCCAATCGTGAGAAGATG
               ** ******** ** ** ** ***** ** ** **************  * ** ******

Drome Act5c    ACCCAGATCATGTTCGAGACCTTCAACACACCCGCCATGTATGTGGCCATCCAGGCTGTG
Hypdu actin    ACCCAGATCATGTTCGAGACATTCAACACCCCCGCCATGTACGTCGCGATTCAGGCTGTG
Hypkl actin    ACTCAGATCATGTTCGAGACATTCAACACCCCCGCCATGTACGTCGCGATTCAGGCTGTG
Ramob actin    ACCCAGATCATGTTCGAGACCTTCAACACCCCGGCCATGTACGTTGCCATCCAGGCCGTG
Macsp actin    ACCCAGATCATGTTCGAGACCTTCAACACCCCCGCCATGTACGTGGCCATCCAGGCTGTG
               ** ***************** ******** ** ******** ** ** ** ***** ***

Drome Act5c    CTCTCGCTGTACGCCTCCGGTCGTACCACCGGTATCGTTCTGGACTCCGGCGATGGTGTC
Hypdu actin    CTCTCCCTGTACGCGTCCGGTCGTACCACCGGTATCGTGCTGGACTCTGGTGATGGTGTC
Hypkl actin    CTCTCCCTGTACGCGTCTGGTCGTACCACAGGTATCGTTCTGGATTCTGGTGATGGTGTC
Ramob actin    CTGTCCCTGTATGCGTCTGGTCGTACCACCGGTATCGTGCTGGACTCTGGTGATGGTGTC
Macsp actin    CTGTCCCTGTACGCCTCTGGTCGTACCACTGGTATCGTGTTGGATACTGGTGATGGTGTG
               ** ** ***** ** ** *********** ********  ****  * ** ********

Drome Act5c    TCCCACACCGTGCCCATCTACGAGGGTTATGCCCTTCCCCATGCCATCCTGCGTCTGGAT
Hypdu actin    TCCCACACTGTCCCCATCTACGAAGGTTATGCTCTGCCTCACGCCATCCTCCGTCTGGAT
Hypkl actin    TCCCACACTGTCCCGATCTACGAAGGTTATGCTCTGCCCCATGCCATCCTCCGTCTGGAT
Ramob actin    AGCCACACTGTGCCCATCTATGAAGGTTATGCTCTGCCCCATGCCATTCTCCGTCTGGAT
Macsp actin    TCTCACACTGTGCCCATCTACGAAGGCTATGCCCTGCCTCACGCCATCCTGCGTCTGGAT
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Fig. 1. Comparison of the actin sequence of *Drosophila melanogaster* ("Drome Act5c") with actin fragments of the eutardigrades *Hypsibius dujardini* ("Hypdu actin"), *Hysibius klebelsbergi* ("Hypkl actin"), *Ramazzottius oberhaeuseri* ("Ramob actin") and *Macrobiotus* sp. ("Macsp actin"). An untranslated leader of the *H. dujardini* sequence was removed. The primers we used are underlined and written above the sequences at their correct positions. They are in 5' to 3' direction Actup, ActU235, ActL667 und Actdown. Matching positions are marked with a star. **a**: Nucleic acids. **b**: Amino acids. Continued.

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Drome Act5c    CTGGCTGGTCGCGATTTGACCGACTACCTGATGAAGATCCTGACCGAGCGCGGTTACTCT
Hypdu actin    TTGGCCGGTCGCGACTTGACTGACTACTTGATGAAGATCCTGACTGAGCGCGG-TACAGC
Hypkl actin    TTGGCCGGTCGCGACTTGACTGACTACTTGATGAAGATCCTGACTGAACGCGGTTACAGC
Ramob actin    TTGGCCGGTCGCGATCTGACTGACTATCTGATGAAGATTCTGACTGAACGTGGTTACAGC
Macsp actin    CTGGCTGGTCGCGATCTGACTGACTACCTCATGAAGGTCCTGACTGAGCGTGGCTACTCC
                **** ********  **** *****  * ****** * ***** ** ** ** ***

Drome Act5c    TTCACCACCACCGCTGAGCGTGAAATCGTCCGTGACATCAAGGAGAAGCTGTGCTATGTT
Hypdu actin    TTCGTCACAAC-GCT---------------------------------------------
Hypkl actin    TTCGTCACAACCGCTGAACGAGAGATAGTCCGTGACATCAAGGAAAAGCTCTGCTACGTT
Ramob actin    TTCGTCACAACCGCTGAACGCGAAATTGTCCGTGACATCAAGGAGAAACTCTGCTACGTC
Macsp actin    TTCACCACCACCGCCGAGCGCGAAATCGTCCGTGACATCAAGGAGAAGCTGTGCTACGTC
               ***  *** ** **

                     GAYTTYGARCARGARATGGC
Drome Act5c    GCCCTCGACTTTGAGCAGGAGATGGCCACCGCTGCCAGCAGCTCCTCGTTGGAGAAGTCC
Hypdu actin    ------------------------------------------------------------
Hypkl actin    GCTCTCGACTTCGAGCAGGAAATGGCCACCGCTGCTGCATCCTCCTCCCTGGAAAAGAGC
Ramob actin    GCCCTGGACTTCGAACAAGAAAT-------------------------------------
Macsp actin    GCCCTTGACTTCGAACAAGAAATGGCA---------------------------------

Drome Act5c    TACGAGCTGCCCGACGGACAGGTGATCACCATCGGCAACGAGCGTTTCCGCTGCCCCGAG
Hypdu actin    ------------------------------------------------------------
Hypkl actin    TACGAACTTCCCGACGGTCAAGTCATCACCATCGGAAACGAGCGATTCCGATGCCCTGAG
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------

Drome Act5c    GCCCTGTTCCAGCCCTCGTTCTTGGGAATGGAGGCTTGCGGCATCCACGAGACCACCTAC
Hypdu actin    ------------------------------------------------------------
Hypkl actin    GCTCTCTTCCAACCCAGCTTTATCGGTATGGAGTCGTGCGGTATCCATGAGACCACCTAC
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------

Drome Act5c    AACTCCATCATGAAGTGTGATGTGGATATCCGTAAGGATCTGTATGCCAACACCGTGCTG
Hypdu actin    ------------------------------------------------------------
Hypkl actin    AACTCGATCATGAAGTGCGACATTGATATCCGTAAGGATCTGTACGCCAACACTGTGTTG
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------

Drome Act5c    TCCGGTGGCACCACCATGTACCCTGGCATCGCCGACCGTATGCAGAAGGAGATCACCGCC
Hypdu actin    ------------------------------------------------------------
Hypkl actin    TCCGGCGGTACCACCATGTACCCTGGTATTGCCGATCGCATGCAGAAGGAGATCACCGCC
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------

Drome Act5c    CTGGCACCGTCGACCATGAAGATCAAGATCATTGCCCCGCCAGAGCGCAAGTACTCTGTC
Hypdu actin    ------------------------------------------------------------
Hypkl actin    CTTGCGCCCAGCACAATGAAGATCAAAATCATCGC-------------------------
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------

                                             ACNTTYCARCARATGTGGATH
Drome Act5c    TGGATCGGTGGCTCCATCCTGGCTTCGCTGTCCACCTTCCAGCAGATGTGGATCTCCAAG
Hypdu actin    ------------------------------------------------------------
Hypkl actin    ------------------------------------------------------------
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------

Drome Act5c    CAGGAGTACGACGAGTCCGGCCCCTCCATTGTGCACCGCAAGTGCTTCTAA
Hypdu actin    ---------------------------------------------------
Hypkl actin    ---------------------------------------------------
Ramob actin    ---------------------------------------------------
Macsp actin    ---------------------------------------------------
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Fig. 1. Continuation.

b

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Drome Act5c    MCDEEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQ
Hypdu actin    M-EDEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQ
Hypkl actin    ------------------------------------------------------------
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------


Drome Act5c    SKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM
Hypdu actin    SKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM
Hypkl actin    -----------------------EKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM
Ramob actin    ------------------NWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM
Macsp actin    ------------------NWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM
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Drome Act5c    TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLD
Hypdu actin    TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLD
Hypkl actin    TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLD
Ramob actin    TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLD
Macsp actin    TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDTGDGVSHTVPIYEGYALPHAILRLD
               *********************************** ************************

Drome Act5c    LAGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKS
Hypdu actin    LAGRDLTDYLMKILTERG---TASSQR---------------------------------
Hypkl actin    LAGRDLTDYLMKILTERGYSFVTTAEREIVRDIKEKLCYVALDFEQEMATAAASSSLEKS
Ramob actin    LAGRDLTDYLMKILTERGYSFVTTAEREIVRDIKEKLCYVALDFEQE-------------
Macsp actin    LAGRDLTDYLMKVLTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMA-----------
               ************ *****        *

Drome Act5c    YELPDGQVITIGNERFRCPEALFQPSFLGMEACGIHETTYNSIMKCDVDIRKDLYANTVL
Hypdu actin    ------------------------------------------------------------
Hypkl actin    YELPDGQVITIGNERFRCPEALFQPSFIGMESCGIHETTYNSIMKCDIDIRKDLYANTVL
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------


Drome Act5c    SGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISK
Hypdu actin    ------------------------------------------------------------
Hypkl actin    SGGTTMYPGIADRMQKEITALAPSTMKIKII-----------------------------
Ramob actin    ------------------------------------------------------------
Macsp actin    ------------------------------------------------------------


Drome Act5c    QEYDESGPSIVHRKCF
Hypdu actin    ----------------
Hypkl actin    ----------------
Ramob actin    ----------------
Macsp actin    ----------------
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Fig. 1. Continuation.

These differences appear rather high in view of the fact that actin exhibits 80.2% sequence conservation at the gene level between *Homo sapiens* and *Saccharomyces cerevisiae* Meyen & Hansen, 1883, and 95% conservation of the primary structure of the protein product (e.g. Vandekerckhove *et al*. 2003).

By screening our cDNA library with an actin fragment as a probe we have found eight different positive clones in the GenBank so far. Therefore, we expect to get the complete coding sequence of the gene as well as the 3`and 5`UTR of the predominant isoforms.

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