The Journal of Experimental Biology 203, 2747–2756 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JEB2673

TEMPORAL AND SPATIAL EXPRESSION OF THE CELL-CYCLE REGULATOR *CUL-1* IN *DROSOPHILA* AND ITS STIMULATION BY RADIATION-INDUCED APOPTOSIS

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Accepted 20 June; published on WWW 22 August 2000

Summary

Cul-1 protein is part of the ubiquitin ligase complex that is conserved from yeast to humans. This complex specifically marks cell-cycle regulators for their subsequent destruction. Two null mutations of the *cul-1* gene are known, in budding yeast and in nematodes. Although in both these organisms the *cul-1* gene executes essentially the same function, the manifestation of its lack-of-function mutations differs considerably. In yeast the mutation causes arrest at the G_1/S-phase transition, whereas in nematodes excessive cell divisions occur because mutant cells are unable to exit the mitotic cycle. We isolated *cul-1* orthologues from two model organisms, *Drosophila melanogaster* and mouse. We show that the *Drosophila* full-length *cul-1* gene restores the yeast mutant's inability to pass through the G_1/S-phase transition. We also characterize expression of this gene at the transcript and protein levels during *Drosophila* development and show that *cul-1* gene is maternally supplied as a protein, but not as an RNA transcript. Zygotic transcription of the gene, however, resumes at early stages of embryogenesis. We also found an increase in *cul-1* transcription in cultured cells treated with a lethal dose of γ-irradiation.

Key words: *Drosophila melanogaster*, cell cycle, *cul-1* gene, cullin, ubiquitin.

Introduction

In eukaryotic cells, passage through different stages of the cell cycle is controlled by a variety of processes including the synthesis or activation of regulatory proteins as well as the destruction of others. Moreover, cell-cycle transitions are triggered by degradation of specific proteins (Cohen-Fix and Koshland, 1997; Lyapina et al., 1998), one of which is associated with ubiquitin-dependent proteolysis of cell-cycle regulators by the proteasome complex (Deshaies, 1995). Cell regulators targeted for destruction, including phosphorylated cyclins, are labeled by specific ubiquitination with the help of evolutionarily conserved ubiquitin ligase complexes known as SCFs (Lyapina et al., 1998). Studies of these complexes in budding yeast and human cell cultures revealed that they contain Cdc53 and Cul-1 proteins, respectively (Feldman et al., 1997; Skowyra et al., 1997). Both proteins belong to a family, the cullins, of which there are at least five members in nematodes and six in humans (Kipreos et al., 1996). Though cullins share a significant homology, probably reflecting their common biochemical properties, the functions of most, except for Cul-1 and Cdc53, have not been identified. Mutations of both *cdc53* in budding yeast and *cul-1* in nematode affect cell-cycle control and are lethal, indicating that other members of the cullin family are then unable to perform their function.

The high level of similarity between the yeast and nematode proteins suggests that their genes are orthologues, but mutations in their genes in these two organisms are manifested differently. While the *cdc53* gene mutation causes developmental arrest at the G_1/S-phase transition in yeast, the nematode *cul-1* mutant displays hyperplasia in all postembryonic tissues because affected cells are unable to exit the mitotic cycle and thus divide excessively. This latter observation led Kipreos et al. (1996) to propose that Cul-1 is a negative repressor of proliferation in nematodes. The observed phenotypic difference between yeast and nematode mutants necessitates further investigation into the role of *cul-1* orthologues in other organisms.

Here we describe the isolation and characterization of *cul-1* genes in two popular model organisms: *Drosophila melanogaster* and mouse. We also show that the *Drosophila cul-1* gene is able to restore the ability of the *cdc53* defective yeast mutant to pass through the G_1/S-phase transition, showing these two genes are true orthologues. We also characterize the temporal and spatial patterns of *cul-1* gene expression at both the transcript and protein levels during *Drosophila* development. Moreover, we show increased *cul-1* transcription when cell proliferation is inhibited and apoptosis is induced by γ-irradiation. These data suggest *Drosophila cul-1* is probably a negative repressor of cell division, as previously

observed in nematodes. We conclude that in higher multicellular eukaryotes Cul-1 participates in the regulation of several different phases of the cell cycle.

Materials and methods

cDNA libraries and sequencing

A *Drosophila* central nervous system-specific cDNA library in λgt10 was prepared as previously described (Li et al., 1995). A mouse cDNA λgt10 library, prepared from day-8.5 mouse embryos, was kindly provided by Dr S. Agulnik (Princeton University) (Agulnik et al., 1996). The libraries were plated on *E. coli* strain NM514. The *Drosophila* library was screened with a 560 bp *Eco*RI/*Sal*I fragment of cDNA encoding *Galleria mellonella* neuropeptide sericotropin (Kodrik et al., 1995) to screen for neurospecific genes. This screen identified the *Drosophila cul-1* cDNA. The 1.8 kb *Eco*RI/*Hin*dIII fragment from this cDNA was then used to screen the mouse library. The cDNA probes fragments were ^{32}P-labelled with Random Primed DNA Labeling Kit (USB) and hybridized to 2×10^5–3×10^5 plaques transferred to Hybond-N membranes (Amersham). Hybridization was performed in 5× SSPE (0.9 M NaCl, 0.05 M NaH_2PO_4, 5 mM EDTA, pH 8.0), 5× Denhardt's solution, 0.1 % SDS and 100 μg ml^{-1} calf thymus DNA at 50 °C for the *Drosophila* library, and at 55 °C for the mouse library. After a 1 h prehybridization, the membranes were incubated with heat-denatured probes for 12–16 h and then washed twice at the hybridization temperature in 1× SSPE, 0.1 % SDS, and twice in 0.1×SSPE, 0.1 % SDS. Positive clones isolated were sequenced using Sequenase Version 2.0 kit (USB).

Sequence analysis

Nucleotide sequences of the isolated clones and their open reading frames (ORFs) were searched against the GenBank database using the BLAST program (Altschul et al., 1997). Protein alignments and phylogenetic tree construction were performed with the aid of the DNASTAR software package.

Complementation analysis

The *S. cerevisiae* KJB-1 (*MAT***a** *ade2 trp1 ura3-52 mip1::URA3 cdc53-2*) strain carrying the temperature-sensitive mutation of *cdc53* gene was kindly provided by Dr M. Goebl (Indiana University). To prepare constructs containing the *Drosophila cul-1* gene we generated a polymerase chain reaction (PCR) fragment using the cDNA clone as a template and two primers. The upstream primer 5′-CCCGAATTCGCCGCCAGAATGAACCGCTC contained an *Eco*RI site and the ATG start site (underlined), while the downstream primer 5′-CGCGGGCCCATTTAACAAAAA-CTTAGGC contained the stop codon (underlined) and a *Sma*I site. The PCR fragment containing the entire *Drosophila cul-1* ORF (2.6 kb) was cloned into an *Eco*RI/*Sma*I-linearized vector pUC57. To ensure that no PCR-induced mutations were in the final construct a portion (2220 bp) of the DNA obtained from the PCR, between the unique *Eco*RV and *Xba*I sites, was exchanged with the corresponding wild-type fragment. The flanking regions were then sequenced to ensure that no mutations were present in these regions. The 2.35 kb *Eco*RI/*Sma*I fragment containing the full-length *cul-1* gene ORF was then cloned into the corresponding sites of pBDGal4 (Stratagene), producing a hybrid protein, which consisted of the GAL4 binding domain and the Cul-1 protein. Similarly, C-terminal truncated protein derivatives of Cul-1 were prepared using the 2.00 kb *Eco*RI/*Sal*I and 1.82 kb *Eco*RI/*Pst*I fragments. All three constructs were used for transformation of the KJB-1 yeast strain by the lithium acetate procedure (Gietz and Woods, 1994).

Northern blot analysis

RNA was extracted from whole insects or cultured cells with TRIzol Reagent (Gibco BRL), electrophoresed on formaldehyde 1 % agarose gels, and transferred onto Hybond-N membranes (Amersham). The 1.8 kb *Eco*RI/*Hin*dIII fragment of *Drosophila cul-1* cDNA was radiolabeled and used as a probe. Hybridization was conducted essentially as described above for the cDNA library screenings except that it was performed at 65 °C. Hybridization membranes were analyzed using a Personal Molecular Imager (Biorad) with Quantity One software package.

In situ hybridization

For chromosomal localization of the *cul-1* gene, the digoxygenin (DIG)-labeled 1.8 kb *Eco*RI/*Hin*dIII cDNA fragment was hybridized to salivary gland polytene chromosomes according to the method of Hankeln et al. (1994). The *cul-1* gene was mapped using polytene chromosomes of the Oregon R strain and the deletion mutants *Df(2R)ca53/+*, *Df(2R)ca58/+* and *Df(2R)cn89e1/+* (Alexandrov and Alexandrova, 1991). Detection of the hybridization signal was performed using anti-DIG-alkaline phosphatase conjugate (Boehringer Mannheim) according to the manufacturer's protocol.

Localization of *cul-1* transcripts during embryogenesis and oogenesis was performed using antisense and sense *cul-1* RNAs, which were synthesized by SP6 and T7 RNA polymerase, respectively. DIG-labeled probes were hybridized as described (Mason et al., 1994). Dissected ovaries and dechorinated eggs were fixed in 4 % paraformaldehyde. Hybridization of labeled probes was performed at 65 °C in 50 % formamide for 24–36 h. Washes were carried out at the same temperature for 2 days. Oogenesis and embryo development were staged according to King (1970) and Bownes (1982), respectively.

Antibody production, western blotting and immunohistochemistry

To prepare antibodies against the Cul-1 *Drosophila* protein, the 675 bp *Sac*I/*Sal*I fragment of the *Drosophila cul-1* cDNA clone was inserted into the pQE30 vector. Isopropyl β-D-thiogalactoside (IPTG) induction of protein expression and Ni^{2+}-agarose purification of the fused poly(His)-containing protein were done according to the manufacturer's protocol

(Qiagen). The purified protein was used for antiserum production as described (Filippova et al., 1998). Western blotting was performed as described (Filippova et al., 1998). Immunohistochemistry was conducted using whole mounts of embryos or selected organs from postembryonic developmental stages (Filippova et al., 1998).

γ-irradiation of Drosophila *Kc 167 cells*

Drosophila Kc 167 cells, kindly provided by Dr L. Cherbas (Indiana University), were cultured in M3 medium with 3 % fetal bovine serum at 25 °C. Cells were seeded at 1×10^6 cells ml^{-1} and grown for 24 h. This semiconfluent culture was irradiated with a dose of 28 krad. 2 ml cell culture samples were collected at the indicated time, washed twice with PBS and used in a TUNEL assay (Boehringer Mannheim) or for total RNA extraction using TRIzol Reagent (Gibco BRL). Serial dilutions of total RNA samples were used for RT–PCR analysis of *reaper* gene expression utilizing an Access PCR kit (Promega). Primers CTACCAGTTGTGTA-ATTCCGAACGAG and TTGGACTGGACTATTGGTTTT-CCCCG produced a *reaper*-specific fragment 293 bp long. The amount of RNA was also tested using actin-specific primers GTCGACAATGGCTCCGGCATGTGCA and TCAGCGCG-CGTTAGAAGCACTTGCGGT.

Results

Sequence analysis of Drosophila *and mouse* cul-1 *homologues*

While screening a brain *Drosophila* cDNA library for neurospecific clones, we isolated a clone with a 2.8 kb insert that hybridized to sericotropin cDNA probe from the moth *Galleria mellonella* (Kodrik et al., 1995) at low stringency. Sequence analysis revealed the presence of an open reading frame (ORF) 2419 bp long capable of encoding a protein of 773 amino acid residues with a calculated molecular mass of 89.4 kDa. The GenBank accession number of the sequence is L41642. The protein had no signal peptide or transmembrane regions, but contained a potential bipartite nuclear targeting sequence at amino acid residues 704–721 (Dingwall and Laskey, 1991). Both of these features suggested that the cDNA clone encoded a nuclear cytosolic protein. GenBank BLAST analysis using this deduced protein sequence identified several related proteins belonging to the cullin family, a novel family of cell-cycle regulation proteins recently identified in higher eukaryotes (Kipreos et al., 1996). A BLAST search using *G. mellonella* sericotropin DNA and protein sequences showed only low levels of similarity to cullins, and this homology was restricted to a small region. Therefore, we believe that sericotropin, which belongs to the lipocalin superfamily, is functionally unrelated to the cullin family of proteins.

Screening of an embryonic mouse cDNA library with the *Drosophila cul-1* cDNA clone as a probe under mild hybridization conditions led to the isolation of a 3.0 kb cDNA clone, encoding a 776-residue protein, which is highly similar to product of the human *cul-1* gene. Its GenBank accession number is AF176910. Phylogenetic analysis confirmed that the *Drosophila* and mouse gene products are homologous to cullins, with the highest similarity to *cul-1* orthologue products of this gene family (Fig. 1A). Fig. 1B displays the alignment of *Drosophila* and mouse protein sequences designated as dmcul-1 and mmcul-1, respectively, with the human cullin-1 protein (hscul-1) and its yeast orthologue Cdc53. The human and mouse proteins are 98.9 % identical, the major difference being the presence of a 24-residue stretch in the mouse protein, amino acids 59–83, which is absent in the human gene. The *Drosophila* protein has 59 % similarity to both human and mouse Cul-1 proteins and 24.7 % to the yeast Cdc53. These data suggest that we isolated the *Drosophila* and mouse homologues of the *cul-1* gene.

The full-length Drosophila cul-1 *complements the* cdc53 *mutation in yeast*

To confirm the phylogenetic analysis suggesting that *Drosophila cul-1* is an orthologue of the yeast *cdc53* gene, we complemented the *cdc53* yeast conditional mutant with the *Drosophila cul-1* gene. The yeast KJB-1 strain has a temperature-sensitive *cdc53-2* mutation that causes developmental arrest at 36 °C (Mathias et al., 1996). We transformed the KJB-1 strain using constructs having either the full-length *Drosophila cul-1* gene or its truncated forms. *Drosophila cul-1* genes that gave C-terminal truncated proteins (Dmcul-1ΔPst, truncated at amino acid 604, or Dmcul-1ΔSal, truncated at amino acid 664) did not change the mutant phenotype at 36 °C. In contrast, full-length *Drosophila* cul-1 protein complemented the mutation, allowing the transformant to propagate at the nonpermissive temperature (Fig. 2).

Temporal expression pattern of Drosophila cul-1 *gene*

The temporal pattern of *Drosophila cul-1* gene transcription was determined using northern and western analyses (Fig. 3). Hybridization of total RNA isolated from whole animals at different developmental stages with the *Drosophila cul-1* cDNA probe revealed the presence of a single approx. 3 kb mRNA band (Fig. 3A). The expression of *cul-1* mRNA was high in 0–4 h embryos, moderate in the older (4–16 h) embryos and very low in the larvae. It rises at the pupal stage and high expression levels were observed in adult females. Western blotting with Cul-1 antibodies also showed the highest concentration of the Cul-1 protein in early embryos. Both protein and transcript levels decrease by the third instar larvae; however, transcript levels show a greater decrease than Cul-1 protein levels (Fig. 3B).

Drosophila cul-1 *gene is expressed during oogenesis and accumulates as a protein in mature oocytes*

The higher level of *cul-1* mRNA observed in adult females when compared to males (Fig. 3A) may be due to its high expression in ovaries. We therefore also monitored the spatial and temporal expression of the *cul-1* gene during oogenesis. *In situ* hybridization using a *cul-1* antisense probe showed the presence of the transcript in nurse cells during most of oocyte

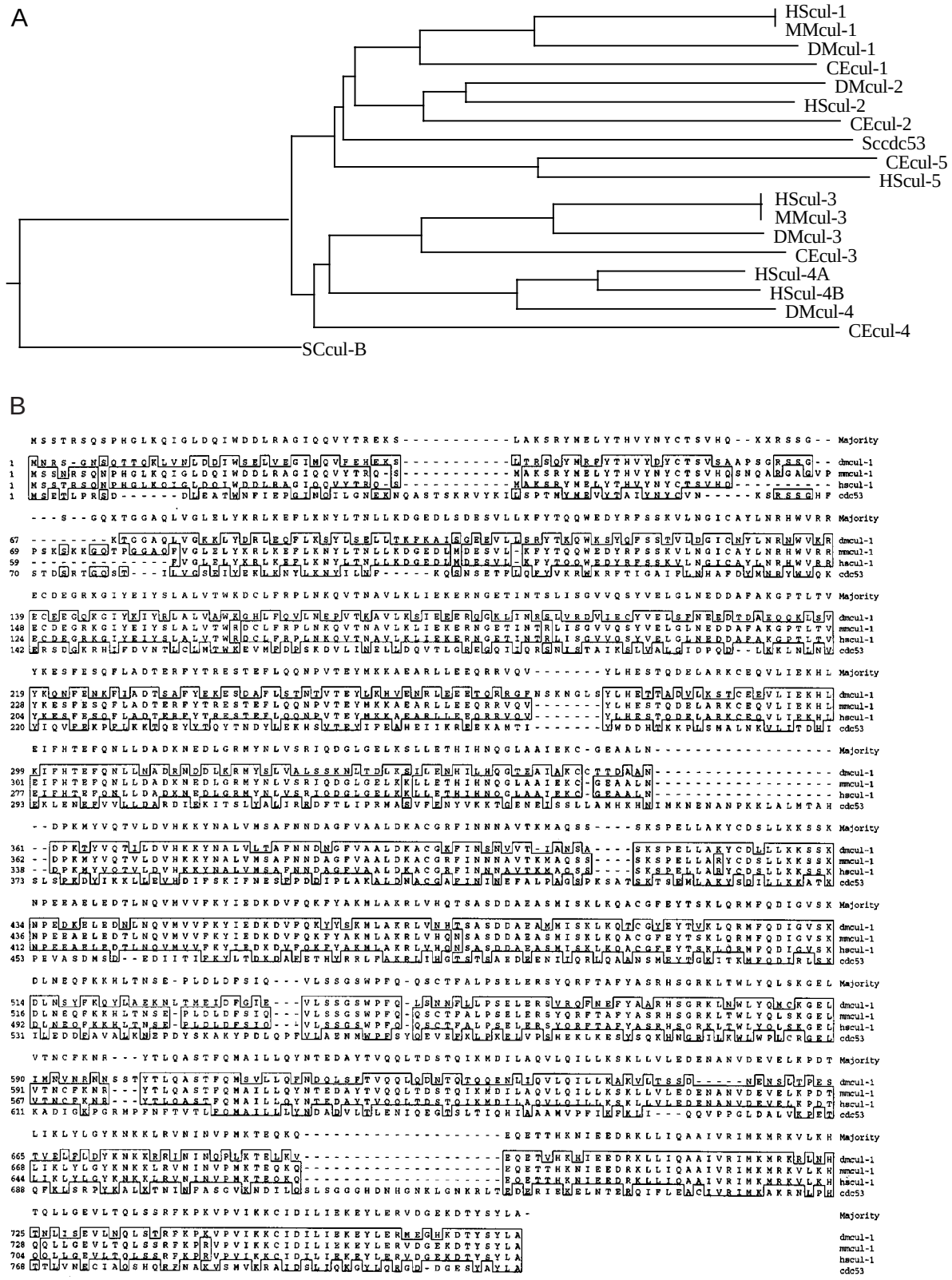

Fig. 1

development. Transcript levels increased progressively as the egg chambers developed and reached maximum levels at ovarian stage 10 (Fig. 4A). At stages 11 and 12, during nurse cell dumping, *cul-1* transcripts passed from the nurse cells into the oocytes. A strong reaction was observed in the ooplasm during nurse cell degeneration, but all detectable *cul-1*

Fig. 1. (A) Phylogenetic tree of some members of the cullin protein family constructed on the basis of sequence alignment. Human cullins are represented by HScul-1, HScul-2, HScul-3, HScul-4A, HScul-4B and HScul-5 proteins (accession numbers AAC36681, AAC50545, NP_003581, AAD45191, AAB67315 and NP_003469, respectively). Nematode cullins include CEcul-1, CEcul-2, CEcul-3, CEcul-4 and CEcul-5 (accession numbers Q17389, Q17390, Q17391, Q17392 and Q23639, respectively). Yeast cullins are shown as Sccdc53 and SCcul-B (accession numbers NP_010150 and NP_011517, respectively). Sequences of *Drosophila* (DMcul-1) and mouse (MMcul-1) Cul-1 proteins are taken from this article (accession numbers L41642 and AF136343, respectively). Sequences of other *Drosophila* (DMcul-2, DMcul-3 and DMcul-4) and mouse cullins (MMcul-3) have accession numbers AAF57224, AAF44933, AAF59135 and AAD36500, respectively. The Cul-1 orthologues are localized in a separate branch. (B) Alignment of Cul-1 protein sequences derived from *D. melanogaster* (dmcul-1), mouse (mmcul-1), human (hscul-1), and *S. cerevisae* (cdc53). Majority represents the consensus sequence of the alignment. Amino acid residues that match the consensus sequence are boxed.

transcripts disappeared abruptly just before the end of nurse cell breakdown. No *cul-1* mRNA was found in any region of stage-14 oocytes or in mature eggs (Fig. 4A).

cul-1 gene transcription during oogenesis was coupled with its translation. Cul-1 antibodies showed that Cul-1 protein is predominantly localized in nurse cells until stage 10 (Fig. 4B). During nurse cell breakdown the Cul-1 protein, just as the *cul-1* mRNA, was transported into oocytes (Fig. 4C). In contrast, however, to the disappearance of the *cul-1* gene transcripts at the last stage of oogenesis, mature unfertilized eggs contained high levels of the Cul-1 protein (Fig. 4D).

The cul-1 *gene actively expression resumes in early embryogenesis*

No *cul-1* transcripts were found in embryos at the beginning of zygotic development (Fig. 5A), but a wave of *cul-1* gene transcription was observed shortly afterwards. The first *cul-1* mRNA was detected at the embryonic stage 3 before syncytial blastoderm formation (Fig. 5B), and transcripts were detected throughout the subsequent cleavage divisions with highest levels observed at stage 4 (Fig. 5C). After cellularization, *cul-1* expression declined progressively during gastrulation and reached a constant low level of expression at stage 8 (Fig. 5D–F). Distribution of mRNA was uniform over the entire embryo during all stages of embryogenesis. The Cul-1 protein level was high at all stages of embryo development starting from the very first divisions (Fig. 6A–D). The protein, as with the mRNA, was distributed evenly throughout the embryo during the early stages of development. At the late stages, Cul-1 protein levels gradually decreased, especially in tissues that have low proliferation rates (Fig. 6D).

The Cul-1 protein is expressed in proliferating tissues during postembryonic development

Various larval and pupal tissues were analysed for the presence of *cul-1* transcripts, which were present in the nuclei of all cell types, whether they were dividing or quiescent (Fig. 7A). The cul-1 protein, however, is preferentially expressed in actively proliferating tissue. For example, high levels of cul-1 protein were found in the gonads (Fig. 7B) and the growing imaginal discs (Fig. 7C).

cul-1 *gene transcription is activated by a lethal dose of γ-irradiation*

To investigate the influence of cessation of cell proliferation on *cul-1* expression in *Drosophila* we irradiated Kc167 cells with a lethal dose of γ-irradiation. Irradiation of a vigorously growing semiconfluent culture with a 28 krad dose caused, within 24 h, an increase in the number of apoptotic cells from approximately 5% in the initial culture to more than 50%. Induction of apoptosis was monitored by the TUNEL assay (data not shown) and expression of the apoptotic gene *reaper* (McCall and Steller, 1997) (Fig. 8A). The irradiation dose that we applied was higher than the lethal 25 krad dose that is commonly used

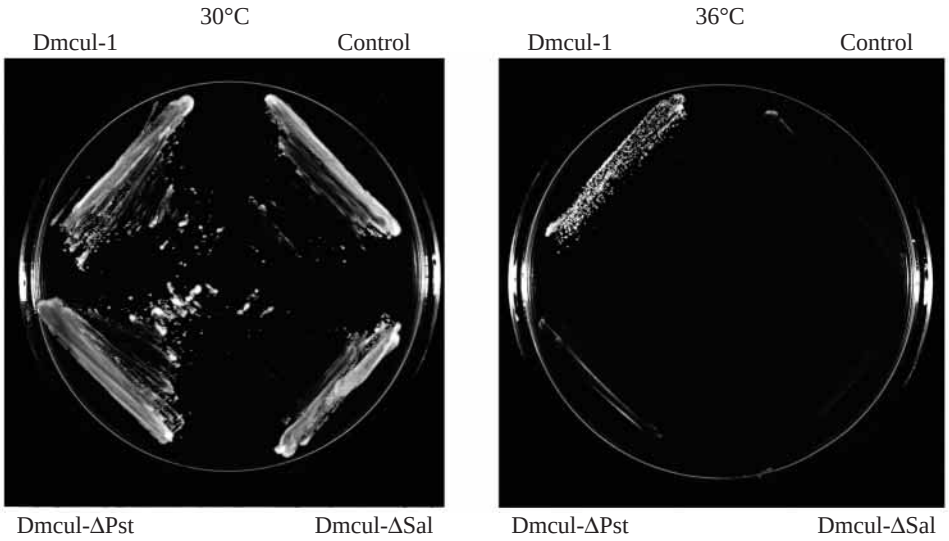

Fig. 2. Complementation analysis of a *cdc53-2* temperature-sensitive mutation in yeast by the *D. melanogaster cul-1* gene and its derivatives. Growth of the *cd53-2* mutant at permissive (30 °C) and restrictive (36 °C) temperatures after transformation with the vector (Control), full-length *Drosophila cul-1* gene (Dmcul-1), and its C-terminal truncated forms Dmcul-ΔPst and Dmcul-ΔSal.

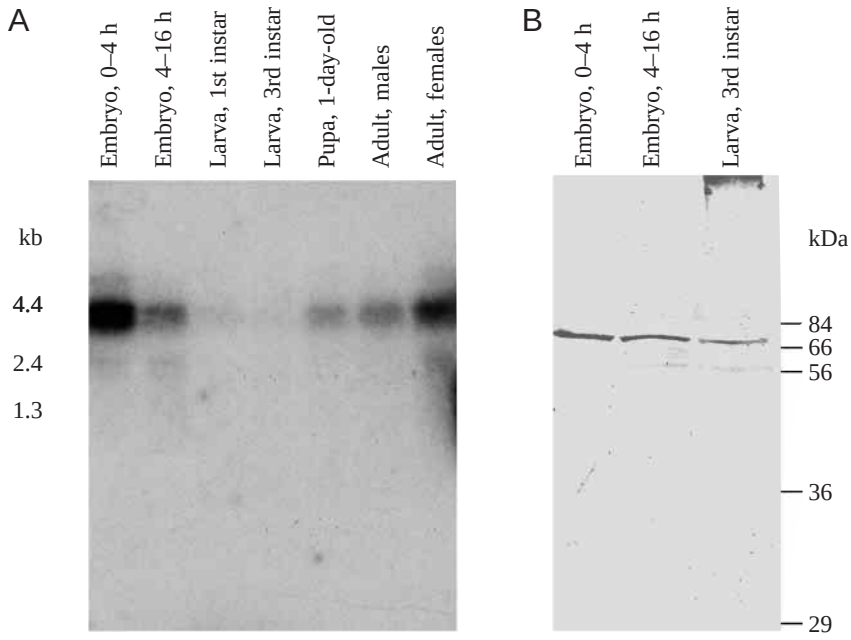

Fig. 3. Expression of the *cul-1* gene at different developmental stages. (A) Northern hybridization of the *cul-1* specific probe to total RNA isolated from early (0–4 h) and late (4–16 h) embryos, first and third instar larvae, and 1-day-old pupae. 1-day-old adult males and females were analyzed separately. (B) Western blot of protein extracts isolated from early and late embryos and the third instar larvae after incubation with *Drosophila* Cul-1 antibody. The positions of markers are shown in each case.

to prepare feeder layer cells (Echalier, 1997). This dose sharply blocks cell division, stops proliferation and induces apoptosis; however, expression of *cul-1* gene, which was monitored by northern analysis of RNA isolated from the irradiated cells, increased twofold at 72 h after irradiation (Fig. 8B).

Chromosomal mapping of the cul-1 *gene in* Drosophila

In situ hybridization was performed using the wild-type Oregon R and the hybrid strains *Df(2R)ca53/+; Df(2R)ca58/+ and Df(2R)cn85e1/+*, which contain deficiencies in the regions 43E6–44B5-9, 43A3–43F6 and 42E4-F1–43E16-F1, respectively (Alexandrov and Alexandrova, 1991). Results of *in situ* hybridization revealed that the deficiencies *Df(2R)ca53* and *Df(2R)ca58* cover the corresponding *cul-1* gene, while *Df(2R)cn85e1* does not (Fig. 9). Thus, using the cytological boundaries of these deletions, the *cul-1* gene maps to position 43F1-6 of the 2R chromosomal arm.

Discussion

The *cul-1* gene in nematodes and its orthologue in yeast, *cdc53*, belong to the *cullin* gene family (Kipreos et al., 1996). Since mutations of *cul-1* and *cdc53* are lethal, the products of these genes must play a vital role and cannot be substituted

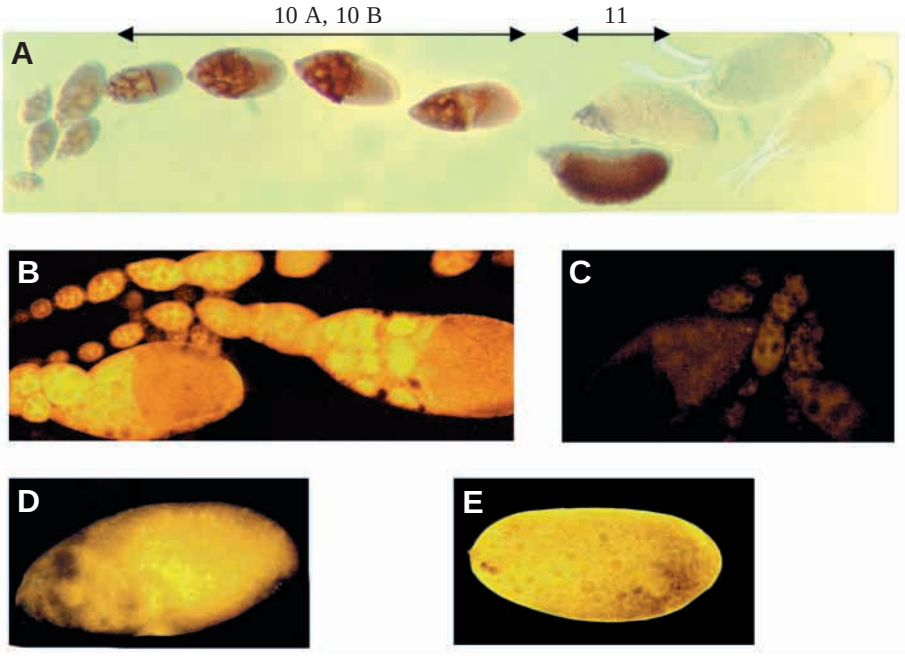

Fig. 4. Expression patterns of the *cul-1* gene during oogenesis. (A) *cul-1* mRNA distribution at different stages of oogenesis. Note the high levels of *cul-1* transcripts at stage 11 and their decline during completion of dumping. No hybridization signal is detected in the fully formed eggs. (B) Cul-1 antibody staining during oogenesis. (C) Staining of ovaries with preimmune serum. (D) Cul-1 antibody staining of an oocyte at the end of vitellogenesis. (E) Cul-1 antibody staining of a laid, unfertilized egg.

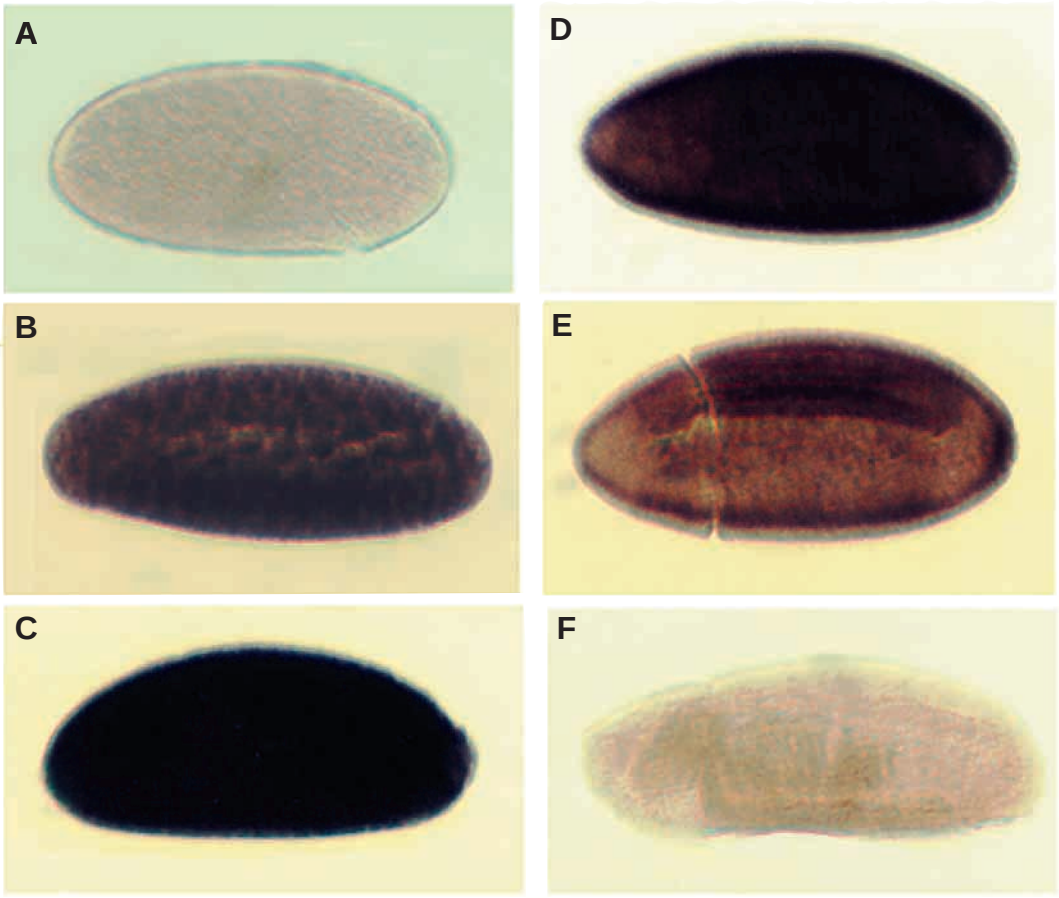

Fig. 5. *Cul-1* gene transcription during embryogenesis. Whole-mount *in situ* hybridization of the *cul-1* specific probe to embryos at (A) stage 2 and (B) stage 3, when the hybridization signal occurs primarily in the perinuclear zones. The highest level of transcription was found at stage 4 (C). Transcription gradually declined starting from cellularization, stage 5 (D), through to the end of embryo development, stage 6 (E) and stage 8 (F).

with other cullins, despite their considerable structural homology. Mutations were shown to affect cell-cycle regulation and *cul-1* and *cdc53* were postulated to share a biochemical mechanism of specific destruction of the cell-cycle regulatory molecules via ubiquitination (Kipreos et al., 1996; Mathias et al., 1996; Willems et al., 1996). The phenotypic effects of mutations in the *cul-1* and *cdc53* genes are, however, surprisingly different. While the yeast *cdc53* mutant is unable to perform the G_1-to-S-phase transition and cease dividing (Mathias et al., 1996), the nematode *cul-1*

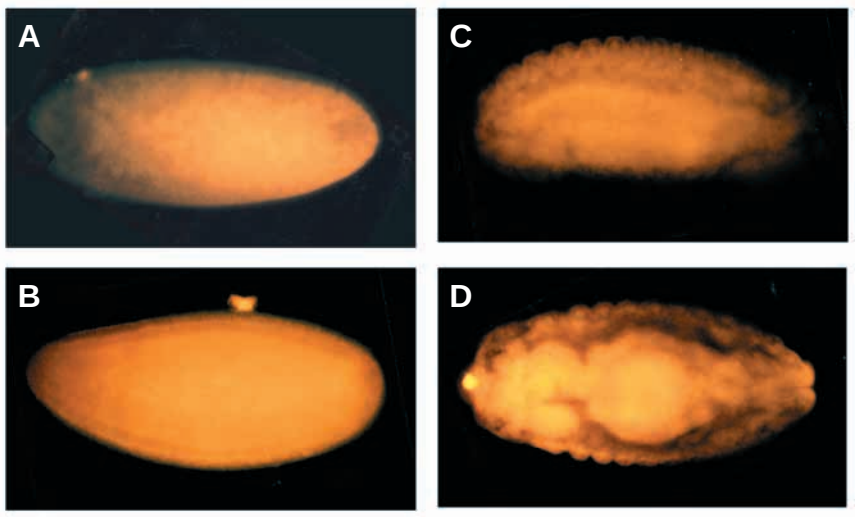

Fig. 6. Localization of Cul-1 protein during embryogenesis. Whole-mount Cul-1 antibody staining of embryos at stage 3 (A), stage 5 (B), stage 9 (C) and stage 12 (D).

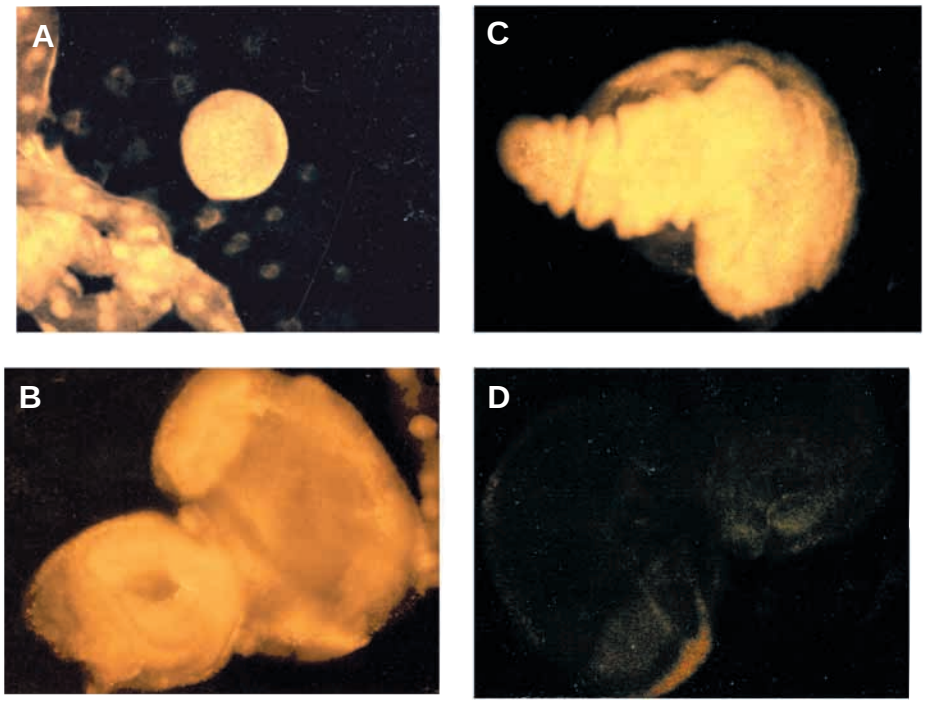

Fig. 7. Cul-1 protein distribution in different tissues. (A) Cul-1 antibody staining of gonads of third instar larva; Malpighian tubules are shown in the bottom right corner. (B,C) Cul-1 staining of the eye-antennal imaginal disc of third instar larva (B) and leg disc of prepupa (C). (D) represents staining of the eye-antennal larval disc with preimmune serum.

mutant displays excessive divisions in postembryonic tissues resulting in hyperplasia, suggesting that the Cul-1 protein is required for exit from the cell cycle (Kipreos et al., 1996). The diverse cell-cycle effects of the orthologous regulators in yeast and nematodes raise the question of how *cul-1* orthologues function in other organisms.

Sequence and phylogenetic analyses of the two genes that we isolated from *Drosophila* and mouse clearly show they are orthologues of *cul-1* and *cdc53*. The mouse protein sequence is practically identical to human Cul-1, except for an additional block of 24 amino acids present at the N terminus. It is noteworthy that this block is also present in the *Drosophila* Cul-1 (Fig. 1B). The level of identity between *Drosophila* Cul-1 and yeast Cdc53 proteins does not exceed 25%, which is comparable to the level of identity between cullin family members from the same species (Kipreos et al., 1996).

The *Drosophila cul-1* gene complements the yeast temperature-sensitive *cdc53* mutant phenotype, as was previously demonstrated for the human *cul-1* gene (Lyapina et al., 1998). Complementation is possible only with the full-length *Drosophila cul-1*, and not with its truncated forms. These results prove that the isolated *Drosophila* gene is a true orthologue of *cdc53*. Since it can control the transition from late G_1 to S phase in yeast, it might also participate in the

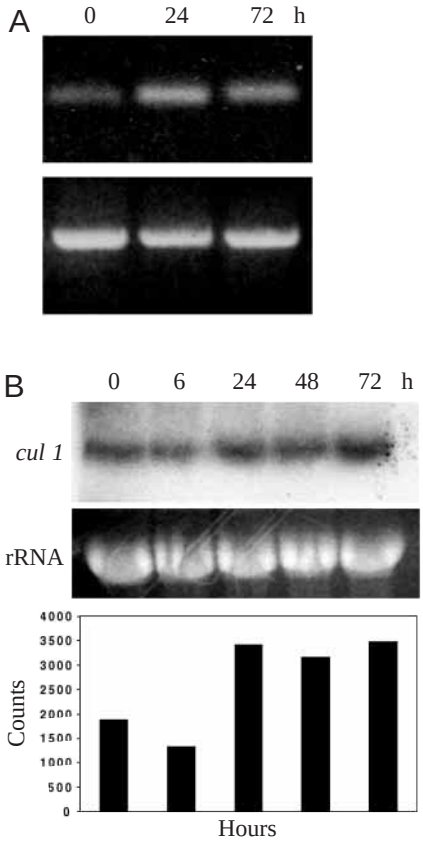

Fig. 8. *cul-1* gene transcription in cultured *Drosophila* Kc167 cells after radiation-induced apoptosis. (A) Induction of the apoptotic gene *reaper* by γ-irradiation. The *reaper*- (upper panel) and actin- (lower panel) specific products obtained by RT-PCR of equal amounts of total RNA isolated from cells before (0 h), and 24 and 72 h after irradiation. (B) Profile of *cul-1* gene transcription in the irradiated cell line. Northern blot analysis was performed using total RNA samples. Amount of *cul-1* transcript was estimated by measuring the radioactivity of the hybridization bands. The data were normalized against the amount of rRNA quantified by densometric scanning (below).

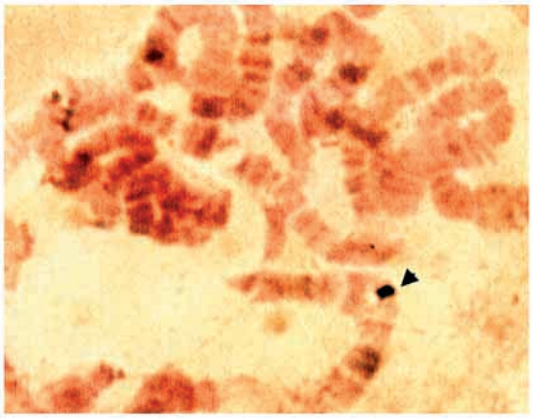

Fig. 9. Localization of the *cul-1* gene on polytene chromosome. DIG-labeled *cul-1* specific probe was hybridized to polytene chromosomes of the heterozygous *Df(2R)ca53/+* mutant. The arrow shows the hybridization signal in the loop derived from the deletion in the 43E6–44B5-9 region.

regulation of this phase transition in *Drosophila*; however, we observed high levels of the Cul-1 protein during the first division cycles after fertilization. Since these divisions proceed without the G_1 phase (Edgar and Datar, 1996), *Drosophila cul-1* gene might be required not only for the G_1-to-S transition, but also for other cell-cycle phase transitions. The Cul-1 protein is known to participate in the SCF complexes associated with ubiquitin-dependent proteolysis. The maternal zygotic transition in *Drosophila* embryos also requires degradation of the maternally derived regulators and thereby inactivation of the maternal cell-cycle control. Thus it is possible that the maternally derived Cul-1 protein is needed for destruction of maternally derived cell-cycle control regulators to change cell-cycle kinetics.

Analysis of Cul-1 protein expression in human cell cultures showed that its level is not periodic throughout cell cycle. It was also shown that Cul-1 protein levels remained high even in serum-deprived cells (Lisztwan et al., 1998). It is not clear, however, whether these unchanged Cul-1 protein levels in G_0–G_1 quiescent cells result from continued gene transcription or stability of the Cul-1 protein. To monitor *cul-1* gene transcription in cells that exit the cell cycle we analyzed *cul-1* mRNA levels in cells that enter apoptosis. During the first 6 h after irradiation we observed a drop in mRNA levels, probably because overall gene expression is sharply inhibited following treatment (Nordstrom et al., 1996). After 24 h of irradiation we found a twofold increase of *cul-1* gene transcription in cells undergoing apoptosis. This response was surprising, and contrary to what would be expected for cell-cycle regulatory proteins. The lethal dose of radiation causes the sharp decline in expression of proteins engaged in cell proliferation. The elevation of *cul-1* expression in irradiated cells suggests that it is required for exit from cell cycle to apoptosis. That Cul-1 is indispensable for the exit to quiescence was shown in *C. elegans* (Kipreos et al., 1996).

Studies of the *cul-1* gene in nematodes failed to detect transcription in early embryos at the four-cell stage but clearly demonstrated an early zygotic gene expression prior to the 24-cell stage (Seydoux and Fire, 1994). Since a maternal effect was observed in the *cul-1* mutants in nematodes, Kipreos et al. (1996) suggested that either the presence of maternally derived transcripts in early embryos was below the threshold level of *in situ* detection, or the Cul-1 protein was stored in oocytes (Kipreos et al., 1996). We therefore specifically analyzed *Drosophila cul-1* gene expression at the mRNA and protein levels during oogenesis and early embryo development. We found high levels of *cul-1* mRNA and the Cul-1 protein in developing oocytes. By the end of egg development, however, *cul-1* mRNA disappears. It is clear that the maternal effect described for the nematode also occurs in *Drosophila* and it is executed via the protein product and not mRNA deposition. The maternal Cul-1 is utilized at early embryonic stages but transcription of the zygotic *cul-1* gene begins before formation of the syncytial blastoderm (Fig. 5B).

The Cdc53 protein is a part of the multiprotein complex that specifically labels and destroys cell regulators through ubiquitination. The same is true for its Cul-1 homologues in higher eukaryotes. We show here, however, that the *Drosophila cul-1* gene product is required for at least two different stages of the cell cycle. This increased complexity of the Cul-1 in multicellular organisms, where stages of quiescence and apoptotic death are parts of programmed development, indicates that the cells of such organisms contain an array of cell-cycle regulators whose breakdown is controlled by Cul-1.

This work was supported in part by a grant from NIH AI32572, the UC Agricultural Experimental Station, and by grant 305/94/0975 from the Grant Agency of the Czech Republic.

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