BIR-1, the Homologue of Human Survivin, Regulates Expression of Developmentally Active Collagen Genes in *C. elegans*

(*Caenorhabditis elegans* / BIR-1 / Survivin / transcription / development)

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Abstract. BIR-1 and Survivin are highly conserved members of the inhibitor of apoptosis protein family that regulate cell division in nematodes and mammals and inhibit apoptosis in mammals. In the *C. elegans* genome, *bir-1* is organized in an operon together with transcription and splicing cofactor CeSKIP (*skp-1*) and is highly expressed during embryogenesis as well as in non-dividing cells during larval development. Previously we have shown that BIR-1 regulates transcription and development and its loss-of-function phenotype overlaps with loss of function of CeSKIP and nuclear hormone receptor CHR3 (NHR-23). Here we searched for genes whose expression is affected by BIR-1 loss of function using whole-genome microarray experiments and identified several collagen genes as candidate targets of *bir-1* inhibition in L1 larval stage. The decreased expression of selected collagen genes in *bir-1*-inhibited larvae was confirmed by quantitative RT-PCR. Next, we generated transgenic lines expressing *bir-1* mRNA under a heat shock-regulated promoter and tested whether *bir-1* overexpression has the potential to augment the expression of genes that showed decreased expression in worms treated with *bir-1* RNAi. Overexpression of *bir-1* resulted in a pronounced increase (2 to 5 times) of the expression of these genes. Our findings support the concept that BIR-1, a protein generally regarded as a mitotic factor, is involved in the regulation of transcription during normal development of *C. elegans* and has a strong ability to affect transcription of developmentally active genes if overexpressed.

Received July 10, 2006. Accepted July 21, 2006.

The work on this project was supported by Grant 301/03/ 0333 from the Czech Science Foundation and by Grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic. This research was supported (in part) by the intramural Research Program of the NIH, NIDDK.

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Abbreviations: IAP – inhibitor of apoptosis protein, Pol II – polymerase II.

Introduction

Regulation of gene transcription is critical for proper development, growth, tissue maintenance and metabolism. The molecular execution of this process depends on a coordinated assembly of the polymerase II (Pol II) basic transcription machinery consisting of general transcription factors (GTFs, TFII A, B, D, E, F and H) and a multi-subunit Mediator complex. This highly dynamic complex comprising up to 30 proteins mediates connection of the basic Pol II transcription complex with transcription factors that recognize specific regulatory sequences in gene promoters – response elements (Malik and Roeder, 2005; Roeder, 2005) and transcription cofactors, a diverse group of proteins that interact with transcription factors. Tissue and metabolic state-specific transcription depends not only on the actual presence of transcription factors (TFs), but also on stimuli coming from the cell membrane and the organism. Posttranslational modifications and spatially restricted availability of transcription factors and cofactors translate to activated or repressed transcription. The ability of proteins to form complexes that activate or repress gene-specific transcription is complemented by modifications that lead to the removal of interacting proteins and the disintegration of protein complexes.

Transcription cofactors form a complex protein network that enhances or represses transcription and are designated depending on the identified role in transcription as coactivators or corepressors. Transcription cofactors may function as activators or repressors depending on the cellular and molecular context and on the structure of the gene promoter.

SKIP, the SKI- interacting protein, is an evolutionarily highly conserved transcription and splicing cofactor affecting transcription regulation by Notch, TGF β and nuclear receptors in metazoan species. We have previously shown that *C. elegans* SKIP (CeSKIP, *skp-1*) is indispensable for normal embryonic and larval development and has an overlapping developmental phenotype with CHR3 (NHR-23), a nuclear receptor that

regulates embryonic and larval development (molting and larval transitions) (Kostrouchova et al.1998; 2001; 2002). Surprisingly, *skp-1* is organized in an operon together with *bir-1*, also a highly conserved protein, which is a member of the inhibitor of apoptosis protein family and is a homologue of the human protein Survivin (Fraser et al., 1999; Speliotes et al., 2000). Inhibitor of apoptosis proteins (IAPs) were originally identified in studies of baculoviruses as proteins able to inhibit apoptosis (Crook et al. 1993; Birnbaum et al. 1994). Contrary to other IAPs, BIR-1 and Survivin are small proteins consisting of two main domains, a BIR domain and a small helical region (Chantalat et al,. 2000; Muchmore et al., 2000; Verdecia et al., 2000). Both regions are likely to mediate interactions with other proteins. Survivin was first shown, in accordance with the function of IAPs, to prevent apoptosis through its ability to bind and inhibit caspases (Ambrosini et al., 1997). Surprisingly, the most prominent function of both survivin and BIR-1 is the regulation of mitotic events, spindle formation and chromosome segregation (Li et al., 1998; Fraser et al., 1999; Speliotes et al., 2000). Survivin has recently been found to regulate the stress response and to interact with the Hsp90 protein (Fortugno et al., 2003; Altieri, 2004;).

Since *bir-1* is expressed together with SKIP (*skp-1*) in non-dividing cells, we studied whether these proteins may be linked functionally. Approximately one third of *C. elegans* genes are organized in operons that often bring functionally linked genes under the regulation of a common promoter (Blumenthal et al., 2002). We showed that *bir-1* inhibition induces phenotypes that are part of loss of function phenotypes of CeSKIP (*skp-1*) and CHR3 (*nhr-23*) (Kostrouchova et al., 2003). We have also shown that *bir-1* inhibition is connected to a decrease in the expression of several transgenes as well as endogenous genes and a decrease of the phosphoacetylated histone H3. In a heterologous transfection system, *bir-1* expression increases the level of phosphoacetylated histone H3 and activates transcription from thyroid hormone-regulated promoters. Its effect is potentiated by the co-expression of human SKIP.

In this study, we searched for genes affected by *bir-1* inhibition during the L1 larval stage using whole genome microarrays. We identified several developmentally important collagen genes as *bir-1* transcriptional targets. Genes whose expression is repressed in *bir-1* inhibited cultures are strongly activated by the forced expression of *bir-1* in transgenic lines expressing *bir-1* from heat shock-regulated promoters. These data support the involvement of BIR-1 in transcription regulation during normal development of *C. elegans* and indicate that non-physiological levels of *bir-1* are able to dramatically affect the transcription profile.

Material and Methods

Strains

The *C. elegans* Bristol N2 strain was used whenever not specifically stated and was maintained as described (Brenner, 1974). The strains SU93- *jcls 1*[*ajm-1: : gfp; unc-29(+)*; *rol-6(su1006*)] expressing AJM: : GFP transgene (Mohler et al., 1998) and JR667- *unc-119 (e2498: : Tcl); wls51* [*unc-119(+); scm: : gfp*] expressing the transgene in the nuclei of seam cells were kindly supplied by the *Caenorhabditis* Genetics Center. The transgenic strain carrying the transgene *pnhr-23: : gfp* that is expressed in epithelial cells including seam cells was prepared as described (Kostrouchova et al., 1998). The lines expressing *bir-1* mRNA from heat shock-regulated promoters were prepared by amplifying *bir-1* cDNA from wild-type N2 worms, which was after subcloning and verification by sequencing re-cloned into the heat-shock promoter vector pPD49.83. Two independent transgenic lines were generated by microinjecting 100 ng/µl of plasmid DNA and a marker plasmid pRF4, *rol-6* (*su1006*) using an Olympus IX70 inverted microscope equipped with a PC-10 Narishige Microinjection System (Narishige, Tokyo, Japan, and Olympus, Prague, Czech Republic).

RNA-mediated interference (RNAi)

The constructs for *bir-1* RNAi were prepared as described (Kostrouchova et al., 2003). The coding region lacking the ATG sequence was amplified, cloned in pCR4-TOPO (Invitrogen, Carlsbad, CA), confirmed by sequencing and re-cloned in the L4440 vector (a kind gift from Dr. A. Fire, Stanford University). The ssRNA was prepared from linearized DNA by *in vitro* transcription reactions using T3 DNA-dependent RNA polymerase (DNA digested by *Sma*I) and T7 DNA-dependent RNA polymerase (DNA digested by *Xba*I) (Promega, Madison, WI). Both sense and antisense RNAs were mixed together at 68°C for 10 min and 37°C for 30 min. dsRNAi was purified by phenol-chloroform extraction, precipitated with ethanol, and the pellet was diluted in water to an approximate concentration of 2 µg/µl. The dsRNAs for RNAi were injected into the gonad of adult hermaphrodites as recommended (Mello and Fire, 1995) and embryos of microinjected animals were scored for *bir-1* loss-of-function mitotic phenotypes.

Large-scale RNA interference

For large-scale culture *bir-1* inhibition, the method of inhibition by feeding of bacteria producing dsRNA to the synchronized cultures was used. *bir-1* cloned in L4440 vector was transformed to HT115 *Escherichia coli* and induced by isopropyl-D galactoside as described (Fire et al., 1998). The control cultures were transformed with L4440 vector carrying the 100 bp long non-specific sequence and a linker sequence that were induced equally as plates used for RNA interference.

Agarose plates were used instead of standard worm plates. The synchronized L1 larval cultures were obtained by harvesting embryos from wild-type hermaphrodites and synchronization by incubation without food overnight in 1x phosphate-buffered saline (PBS). The resulting L1 larvae were collected by centrifugation, the density estimated by counting the number of larvae in a defined volume under the microscope, and equal numbers of larvae were used for paired control-RNA inhibition experiments. The cultures were incubated at 22°C overnight and the developmental stages were determined. No delay in development was observed in any culture. The initial experiments were used to determine the amount of bacterial food that was set to last equally in experimental and control cultures just before the cultures were harvested. Remaining bacterial food was removed by washing the worms three times in water and cultures were left to digest the remaining bacteria during a 30-min incubation time. The resulting cultures were pelleted by short centrifugation at 4°C and frozen. Aliquots of cultures were kept on new plates with induced bacteria for control of the known *bir-1* loss-of-function phenotype that could be detected in later stages.

Total RNA isolation

Synchronized larval cultures of *C. elegans* were grown on 2% agarose-capped plates as described; at appropriate culture times, the plates were washed with water, larvae pelleted by short centrifugation at 4°C and frozen at –80°C. The frozen pellet was resuspended in 0.5 ml of resuspension buffer (0.5% SDS; 5% 2-mercaptoethanol; 10 mM EDTA; 10m M Tris/HCl (pH 7.5) with 12.5 µl of proteinase K (20 mg/ml)). The mixtures were mixed by vortexing for 60 s and incubated for 60 min at 55°C. Proteins were separated by phenol-chloroform extraction and nucleic acids precipitated in ethanol in a final volume of 1350 µl. The 350 µl of water soluble phase was mixed successively with 1 µl of glycogen (20 mg/ml), 35 µl of 10% NaOAc and 950 µl of cold ethanol. The samples were incubated for 30 min on dry ice and centrifuged for 20 min at 4°C at 10,000 *g*. The air-dried pellet was dissolved in water and treated with 1 unit of DNase (Promega) per 1 µg of total RNA for 30 min at 37°C. The DNase was inactivated and extracted by a second round of chloroform extraction and ethanol precipitation and the total RNA was resuspended in DEPC (diethyl pyrocarbonate)-treated autoclaved water. The quality of total RNA stained by ethidium bromide was controlled using agarose gel electrophoresis.

Reverse transcription

Five µg of total RNA were reverse-transcribed with SuperScript II reverse transcriptase (Invitrogen) primed by random hexamers as recommended by the producer.

Microarray experiments

The microarray experiments were kindly done at The Microarray Core Facility, NIDDK, by Dr. M. C. Cam and G. Poy. The standard protocol was used (http://microarray. niddk. nih. gov). The *C. elegans* whole-genome array representing 22,500 transcripts according to GenBank Release 121 were purchased as re-annotated arrays from Affymetrix (Cat. No. 900383 C. elegans Genome Array) (www.affymetrix.com). The data from three independent experiments paired with controls were processed. One experimental set differed from all other data sets and was not used for analysis of data. The data were analysed using the Statistical analysis program (ANOVA) and the Gene Spring computer program. The data are available upon request or will be made accessible at the time of publication at http://udmp.lf1.cuni.cz/DATA1.

The data analysed by the computer program were labelled as Present/Absent (P, A), Increased/Decreased (I, D), No Change (NC), Moderate Increase (MI) and Moderate Decrease (MD). Data were also printed as raw values recorded by the scanner, the P value of data and the P value of change and fold of the change were calculated. The data of selected genes were also evaluated manually by exporting the values to the Excel computer program (Microsoft) and analysed.

Quantitative PCR

The amplicons of two control transcripts, the large subunit of RNA Pol II, *ama-1*, and actin *act-1* and selected transcripts identified in the microarray experiments were designed according to WormBase (Table 1). The selected regions were amplified using PCR, eluted from agarose gel using electrophoresis and the semi-permeable membrane, and the amount of DNA was determined spectrophotometrically. The purified DNA was used to determine a standard curve for each amplified region. Real-time PCR was performed in a PTC200 DNA EngineR thermal cycler equipped with an ALS0296 96-well sample block or PTC 200 Chrome 4 (Bio-Rad, Hercules, CA) and the DyNAmoTM HS SYBR Green qPCR Kit. The modified *Thermus brockians* DNA polymerase was used to prevent extension of non-specifically bound primers during reaction setup. The amplification, including the number of copies in the samples, was characterized using the computer program Opticon MonitorTM Version 3.0. Each sample was analysed by at least three independent analyses. The number of detected copies was normalized according to *ama-1* and *act-1* expression.

The amplification reaction was done in a final volume of 20 µl, containing 500 fmol of primers and 250 ng of total RNA. The cycling consisted of an initial denaturation at 95°C for 15 min, followed by 10 s denaturation at 94°C, annealing at 59°C for 30 s, extension for 30 s at 72°C, and consisted of 45 cycles. The

Table 1. Primers used for quantitative PCR

Gene	Sense primer	5′→ 3′	Antisense primer	5′→ 3′
dpy-2	05-069	gaaatcgcaaacgagtggg	05-070	tgcggatgcctgacaaaa
dpy-4	05-071	ccgccgtctgcttctca	05-072	gttgtaggtagaacgggcgg
dpy-7	05-074	ttggacgaggctcacgaa	05-075	attgttggttgtcggattgag
dpy-8	05-077	atgcgggatttctacgacg	05-079	cggaactgcgtcaccct
dpy-13	05-108	cgtcttctccgttatcgcc	05-109	gcagcatccctcgcatc
col-94	05-093	tcaggcttaccgcttcgt	05-094	tggtggtggggtgattggct
col-125	05-096	cctaccgcttcgttgcct	05-097	ggcagcaagcatcacatcc
col-144	05-099	tctcgttaccgcctctgct	05-100	cggaagatagagttgaatgggtt
col-166	05-102	ccagtctcgcttcttcctcttt	05-103	gcccacctgctttctaccct
col-167	05-106	cgccccaatgctctacaactac	05-107	gcgagaacttgagtgcgggtaa
ama-1	4684	ttccaagcgccgctgcgcattgtctc	4685	cagaatttccagcactcgaggagcgga
act-1	5293	atgtgtgacgacgaggttgccgc	5294	gctcattgtagaaggtgtgatgcc

melting curves were established by the incubation of the final PCR product at 72°C for 10 min and successive denaturation by increasing the temperature from 72°C to 95°C by steps of 1°C for 3 s and a final incubation at 72° C for 10 min.

Results

I. The effect of bir-1 inhibition on L1 larvae

Embryos of wild-type hermaphrodites were prepared using a standard protocol and synchronized L1 larvae were prepared. Synchronized cultures were incubated on a bacterial lawn of control *E. coli* transformed with vector L4440 carrying the cloning site and a non-specific sequence flanked by bacterial promoters on both sides. For *bir-1* inhibition, a 350 bp sequence with the majority of *bir-1* cDNA was used. The production of dsRNA was induced by IPTG in both control and *bir-1*-inhibited cultures. Worms treated with *bir-1* or control RNAi did not differ visibly in the time of L1/L2 molt. Only a slight dpy phenotype was observed in a proportion of *bir-1*-inhibited worms (Fig. 1A). The development of seam cells that divide at the end of L1 stage was monitored in JR667 and SU93 transgenic larvae L1 prepared similarly as wild-type larvae (Fig. 1B, D). These experiments showed that inhibition of *bir-1* by the feeding method does not affect cell divisions of seam cells.

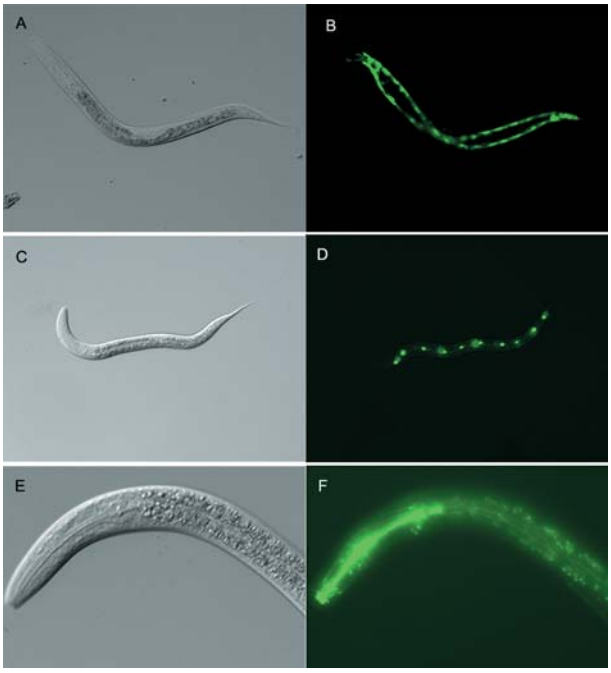

Fig. 1. Morphological analysis of *bir-1*-inhibited larvae. Panel A – L2 larva of transgenic line expressing *pnhr-23: : gfp* (Kostrouchova et al., 1998) treated with *bir-1* RNAi for 24 h shown in Nomarski contrast. A weak dpy phenotype is visible with no other apparent morphological change. Panel B – the same larva that is shown in panel A in fluorescence. Seam cells are visualized by the expression of the transgene. Note the properly formed seam cells in both lateral sides of the larva.
Panel C – L2 larva of JR667 line shown in Nomarski contrast. A weak dpy phenotype is visible in the proximal part of the animal. Panel D – the same larva shown in panel C in fluorescence microscopy. The transgene JR667 marks 10 seam cells during the middle part of L2 larval development, indicating that the dpy phenotype induced by *bir-1* inhibition is independent of seam cell development.
Panel E – the head of an SU93 larva L2 in Nomarski contrast showing a weak dpy phenotype. Panel F – the head of the larva shown in panel E in fluorescent microscopy. The transgene marking the cell membrane of epithelial cells indicates a proper development of seam cells at conditions used in this study.

II. bir-1 interference induces whole-genome transcription changes

To determine the transcription profile by microarrays, three independent experiments were done and evaluated as three control versus *bir-1* RNAi pairs. The statistical analysis labelled the results as Present/Absent (P, A), Increased/Decreased (I, D), No Change (NC), Moderate Increase (MI) and Moderate Decrease (MD). The detection and change P values of the absolute majority of data indicated marginal differences in the analysis of the data. One set of experimental data showed a major problem in evaluation or preparation of the experiment and was eliminated from the analysis. The computer program recognized 67 annotated spots as decreased in both evaluated *bir-1* RNAi experiments and 226 annotated probes as increased in *bir-1* RNAi experiments.

Interestingly, genes recognized as decreased were mostly found in groups of genes that showed highest readings on Affymetrix microarrays. The analysis of values obtained by Affymetrix microarrays showed that the group of 30 gene probes with highest values showed decreased readings in *bir-1* RNAi (Fig. 2A) while the genes with average readings showed both increased and decreased or unchanged values in *bir-1* RNAi-treated cultures (Fig. 2B).

The tendency of decreased readings in RNAi-treated cultures for genes with highest values on Affymetrix microarrays were not observed in our unrelated microarray experiments (not shown).

Genes that were identified as genes inhibited in *bir-1* RNAi included several collagen genes and genes with a known dpy phenotype: *dpy-2*, *dpy-3*, *dpy-4*, *dpy-7*, *dpy-8*, *dpy-9*, *dpy-10*, *dpy-13*, *dpy-15*, *dpy-17*, *col-1*, *col-3*, *col-10*, *col-117*, *col-92*, *col-93*, *col-94*, *col-125*, *col-144*, *col-154*, *col-160*, *col-166*, *col-167, col-169*, genes for several ribosomal proteins: *rps-1, rps-4*, *rpl-2*, *rpl-21* and enzymes (e. g. phosphoheptose isomerase).

Next, we amplified coding regions of selected collagen genes that were identified by microarray analyses and selected genes that were suitable for quantitative PCR according to the amplified fragment (that was confirmed by direct sequencing) and the character of the melting curves. Ten collagen genes fulfilled these criteria and were used for further analysis: *dpy-2*, *dpy-4*, *dpy-7*, *dpy-8*, *dpy-13*, *col-94*, *col-125*, *col-144*, *col-166*, and *col-167*. Manual evaluation of raw data obtained using Affymetrix microarrays indicated a 20 to 40 % decrease in the expression in *bir-1*-inhibited worms (Fig. 2C).

Quantitative RT-PCR confirmed the decreased expression of all evaluated collagen genes (Fig. 3). However, the expression of several collagen genes that were identified by microarrays as genes inhibited in *bir-1* RNAi-treated larvae could not be quantified because unspecific sequences were amplified as indicated by

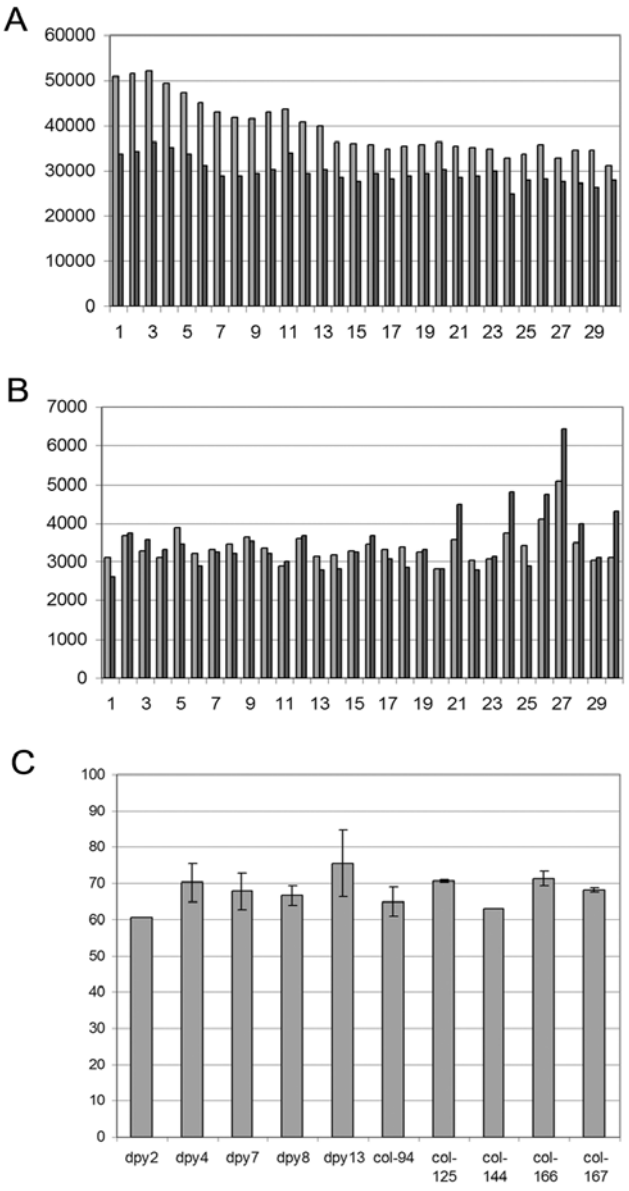

Fig. 2. Whole genome expression analysis by Affymetrix microarrays. Panel A – an average of three evaluated control values of the gene probes with highest reading values (light bars) and an average of values obtained for corresponding gene probes of two *bir-1*-inhibited cultures (dark bars). Note that 30 gene probes with highest reading values show decreased expression in *bir-1*-inhibited cultures. Panel B – an example of values of 30 gene probes that show average reading values (around 3,000 units in Affymetrix arrays). The values obtained for control experiments are similar to the values of cultures that were treated with *bir-1* RNAi (dark bars). Several gene probes show an increase in *bir-1* RNAi-treated cultures. Panel C – raw data obtained by Affymetrix microarrays for selected collagen genes that were evaluated manually in each paired experiment and expressed as percent of control values in the particular experiment and SD estimated. The values show 20 to 40 % decrease in *bir-1*-inhibited cultures.

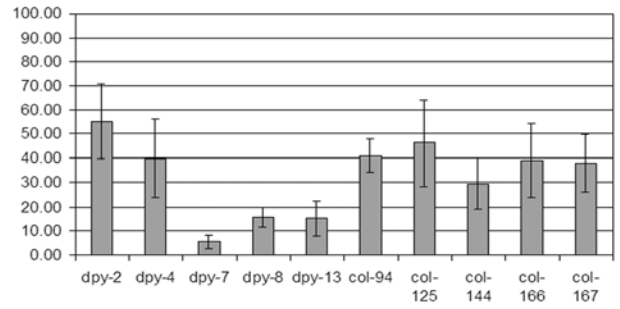

Fig. 3. Validation of data obtained by microarray analysis using quantitative PCR. Average values and SD from a minimum of three independent experiments are shown. Ten selected collagen genes were evaluated and the results are expressed as percent of values found in control experiments. There is a 60 to 90% decrease of the expression of evaluated genes in *bir-1* RNAi-treated cultures.

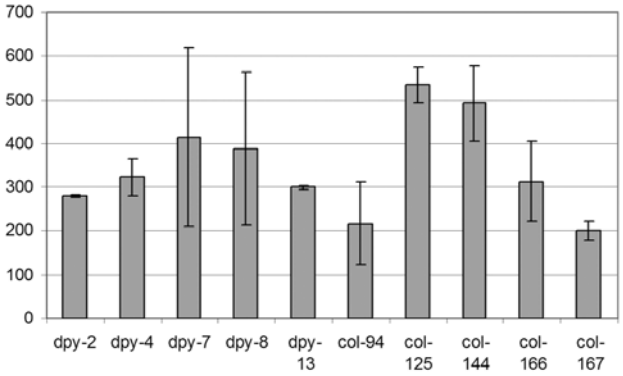

Fig. 4. The expression of selected collagen genes in larvae overexpressing *bir-1* shown as percent of control values. Average values from a minimum of three independent experiments and SD are shown. The expression of all evaluated genes show a pronounced increase in animals with *bir-1* overexpression compared to control wild-type larvae.

multiple bands detected in the final PCR products and by the character of the melting curves.

III. bir-1 overexpression induces a dramatic increase in the expression of collagen genes whose expression is sensitive to bir-1 RNAi during normal development

In order to see whether genes that showed decreased expression in *bir-1*-inhibited larvae are also sensitive to elevated levels of BIR-1, we generated transgenic lines expressing *bir-1* mRNA from a transgene regulated by a heat-shock inducible promoter and confirmed the presence of the transgene by direct sequencing. A short heat-shock induction (30 min at 34°C) strongly induced the expression of all ten examined collagen genes normalized to the expression of actin (*act-1*) (Fig. 4). This induction and an almost identical expression pattern were observed in experiments normalized according to the expression of a large subunit of Pol II, *ama-1*. Interestingly, the expression of transcripts used for the normalization of results (*act-1* and *ama-1*) was elevated in experiments arranged to determine the number of amplified copies in equal amounts of starting material (based on total RNA), indicating that the observed difference in the expression of collagen genes may actually be larger.

Discussion

In this study we show that BIR-1, a homologue of the human cancer-related protein Survivin, regulates expression of several developmentally active collagen genes during the normal development of *C. elegans* and has a strong potential to affect gene expression when expressed at non-physiological levels in transgenic animals.

C. elegans offers a versatile model for studies of transcription regulation in both dividing and non-dividing cells. The development of *C. elegans* proceeds through an embryonic stage and four larval stages to adulthood. Adult *C. elegans* worms have a constant number of somatic cells, 956 in hemaphrodites and 1031 in males. The cell lineages follow a developmental scheme that results in the formation of specialized tissues and cells in a tightly regulated pattern. At the end of the embryonic stage, the newly hatched L1 larva has approximately 550 cells, and the subsequent cell divisions in the L1 stage form an animal that has most cells developed. Only a limited number of cells continue to divide during a narrowly framed time window. Seam cells, specialized epidermal cells that form two lines on each side of the larvae, asymmetrically divide once in each larval stage and each forms one new seam cell that keeps the blast cell character and one epidermal cell that fuses with the main epidermal syncytium. Proper development of seam cells is critical for normal body shape and motility.

Most cells can be regarded as postmitotic in the larval stages of *C. elegans*, and the separation of dividing and non-dividing cells offers a system for studies of protein function related to cell division and postmitotic functions.

BIR-1 and Survivin execute critical functions during cell division: the regulation of spindle formation and the proper separation of chromosomes. The mitotic functions are dramatic and the cell cycle projects to complex cellular events including transcription regulation, which may obscure non-mitotic events. Inhibition of the function of BIR-1 and Survivin leads to mitotic arrests, defects in karyokinesis and cytokinesis, including formation of aneuploid cells and nuclei-free cell bodies (Fraser et al., 1999; Speliotes et al., 2000). The other functions of Survivin include potent inhibition of apoptosis and a newly discovered role in the regulation of the stress response (Fortugno et al., 2003; Altieri, 2004).

Our previous work identified a new function of BIR-1 that is not related to mitotic functions: the regulation of transcription and development in non-dividing cells (Kostrouchova et al., 2003). Structural data that are available for Survivin indicate its wide ability to interact with other proteins. Survivin is a small 123 aa protein that forms a zinc finger BIR domain and a laterally positioned helix. Both domains are likely to effectively bind other proteins including BIR-1 itself as was found in crystallographic studies on Survivin. Survivin was found unexpectedly dimerizing through the region that forms a tip of a triangularly shaped molecule (Chantalat et al., 2000). Survivin and BIR-1 were shown to form complexes with more than one protein. BIR-1 interacts with a small protein CSC-1 (Romano et al., 2003) and INCENP (Romano et al., 2003), and together with both proteins is involved in docking the Aurora B kinase Air-2 to chromosomes (Speliotes et al., 2000).

The binding function of Survivin includes binding of caspases and interaction with mitochondrial proteins. It seems likely that the interaction has inactivating consequences. The antiapoptotic functions seem to have a wide-spread role in most human cancers. The high expression of Survivin is generally linked to a higher malignancy and progression in most cancer types. Both Survivin and BIR-1 were shown to reside both in the cytoplasm and in the nucleus. While the cytoplasmic expression of Survivin is reported as an unfavorable criterion in most studied cases, the nuclear expression was found to be related to a better prognosis.

Despite that, Survivin is generally found to be overexpressed in most cancers, its elevated expression in transgenic mice does not induce cancer formation itself but elevates the sensitivity to cancer-promoting events and is connected with changes of gene expression in microarrays.

The structure and potentially multiple protein interaction potential of BIR-1/Survivin suggests that its elevated expression and spatially restricted presence may induce fundamental changes in the availability of regulatory proteins and may change the capacity of interacting proteins to participate in protein complexes. Since BIR-1 is able to bind Air-2 and phosphorylate histones H3 on P10, we hypothesized that kinase docking may be a part of BIR-1's transcriptional role in non-dividing cells. Histone H3 phosphorylation is the critical event for chromatin compaction during mitosis, which is generally connected to the silencing of transcription but may also be linked to transcription activation. Phosphoacetylated histones H3 in the form of K9-Ac S10-P and S10-P K14-Ac were found to be part of transcription activation and it was suggested that phosphorylation of histone H3 may even precede the acetylation and may actually be the starting event in transcription activation (Hauser et al., 2002). Alternatively, both phosphorylation and acetylation events may be functionally separated and centered synergistically but independently to the promoters of genes (Thomson et al., 2001). The phosphorylation of histone H3 may be connected to regulatory events that exceed the modification of chromatin. Phosphoacetylation of histones in nucleosomes of promoters regulated by c-Fos and c-Jun proteins was shown to be part of transcription activation (Clayton et al., 2000).

The role of Survivin in the regulation of gene expression was also observed in mammalian systems. Survivin enhances the level of p21ras mRNA as well as its activated form at the protein level (Temme et al., 2005). Survivin regulates the expression of p53 and its protein family members both at mRNA and protein levels (Wang et al., 2004). The involvement of Survivin in the regulation of gene expression via the up-regulation of SP-1-mediated gene transcription was shown on colon cancer cells (Asanuma et al., 2004).

The results reported here suggest that BIR-1 may be involved in contacts with multiple transcriptionally active proteins. Identification of collagen genes as targets of BIR-1 transcription regulation is in keeping with the phenotype of *bir-1* loss of function inducing the dumpy (dpy) phenotype that is often connected with defects of collagen genes. Nevertheless, the role of BIR-1 in transcription regulation is likely to be more general. As reported here, *bir-1* inhibition repressed transcription of most but not all genes that showed the highest transcriptional activity in microarrays and decreased the values recorded for 67 genes. A larger group of genes comprising 227 members was identified as genes potentially transcriptionally inhibited by BIR-1. Together with the findings that most transgenes and transfected reporters are more transcriptionally active in the presence of BIR-1, this suggests that BIR-1 is likely to activate the ongoing active transcription. Multiple contacts of BIR-1 with transcriptionally active proteins may project to elevated expression of specific genes in *bir-1* RNAi.

In conclusion, the data reported here support the active role of BIR-1 in transcription regulation during *C. elegans* development and indicate that BIR-1 interferes with transcription regulation when expressed at high levels.

Acknowledgement

We thank Dr. Michael Krause for support and advice throughout the work on this project and Dr. Jacob Robbins for critical reading of the manuscript. We thank Dr. Andrew Fire for vector L4440 and host used in RNAi. We thank Dr. Margaret Cam and George Poy for kindly performing the Affymetrix microarray experiments.

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