The lamin B receptor of *Drosophila melanogaster*

Nicole Wagner, Daniela Weber, Sabine Seitz and Georg Krohne*

Division of Electron Microscopy, Biocenter of the University of Würzburg, Am Hubland, 97074 Würzburg, Germany

*Author for correspondence (e-mail: krohne@biozentrum.uni-wuerzburg.de)

Accepted 10 December 2003
Journal of Cell Science 117, 2015-2028 Published by The Company of Biologists 2004
doi:10.1242/jcs.01052

Summary

The lamin B receptor (LBR) is an integral membrane protein of the inner nuclear membrane that has so far been characterized only in vertebrates. Here, we describe the *Drosophila melanogaster* protein encoded by the annotated gene CG17952 that is the putative ortholog to the vertebrate LBR. The *Drosophila* lamin B receptor (dLBR) has the following properties in common with the vertebrate LBR. First, structure predictions indicate that the 741 amino acid dLBR protein possesses a highly charged N-terminal domain of 307 amino acids followed by eight transmembrane segments in the C-terminal domain of the molecule. Second, immunolocalization and cell fractionation reveal that the dLBR is an integral membrane protein of the inner nuclear membrane. Third, dLBR can be shown by co-immunoprecipitations and in vitro binding assays to bind to the *Drosophila* B-type lamin Dm0. Fourth, the N-terminal domain of dLBR is sufficient for in vitro binding to sperm chromatin and lamin Dm0. In contrast to the human LBR, dLBR does not possess sterol C14 reductase activity when it is expressed in the *Saccharomyces cerevisiae erg24* mutant, which lacks sterol C14 reductase activity. Our data raise the possibility that, during evolution, the enzymatic activity of this insect protein had been lost.

To determine whether the dLBR is an essential protein, we depleted it by RNA interference in *Drosophila* embryos and in cultured S2 and Kc167 cells. There is no obvious effect on the nuclear architecture or viability of treated cells and embryos, whereas the depletion of *Drosophila* lamin Dm0 in cultured cells and embryos caused morphological alterations of nuclei, nuclear fragility and the arrest of embryonic development. We conclude that dLBR is not a limiting component of the nuclear architecture in *Drosophila* cells during the first 2 days of development.

Key words: LBR, Sterol C14 reductase, RNAi, Nuclear envelope, Lamin Dm0

Introduction

The nuclear envelope is an essential structure of the eukaryotic cell that is composed of three distinct membrane domains, the outer and inner nuclear membranes, and the wall of nuclear pore complexes. The inner nuclear membrane is structurally and functionally distinct from the other two membrane domains. It contains specific integral membrane proteins (IMPs) that, during interphase, link the membrane to the underlying lamina and the peripheral chromatin (for recent reviews, see Gruenbaum et al., 2003; Dechat et al., 2000; Ye et al., 1998; Collas and Courvalin, 2000; Cohen et al., 2001).

One of the best studied IMPs is the lamin B receptor (LBR) of vertebrates; other IMPs have been extensively reviewed recently (Gruenbaum et al., 2003; Dechat et al., 2000; Collas and Courvalin, 2000; Starr and Han, 2003). The LBR is composed of two major domains. The ~220-amino-acid N-terminal segment is highly charged, contains two globular domains and faces the nucleoplasm, whereas the hydrophobic C-terminal half of the molecule contains eight putative transmembrane segments (Ye et al., 1997) (for review, see Ye et al., 1998).

The nucleoplasmic domain of LBR binds to B-type lamins (Ye and Worman, 1994; Simos and Georgatos, 1992; Meier and Georgatos, 1994; Dreger et al., 2002), DNA (Ye and Worman, 1994; Duband-Goulet and Courvalin, 2000), chromosomes and chromatin (Pyrpasopoulou et al., 1996; Kawahire et al., 1997; Gajewski and Krohne, 1999), and interacts with human chromodomain protein HP1 (Ye and Worman, 1996; Ye et al., 1997). HP1, the core histones H3 and H4, and the LBR form a tight complex in vitro (Polioudaki et al., 2001). The cell-cycle-dependent binding of the LBR to chromatin is regulated by multiple kinases (Takano et al., 2002). The LBR has two non-overlapping inner nuclear membrane targeting signals. One is localized in the nucleoplasmic domain and the second in the first membrane spanning domain (Smith and Blobel, 1993; Soullam and Worman, 1993; Ellenberg et al., 1997). In cell nuclei that do not possess peripheral chromatin (e.g. the amphibian oocyte nucleus), the LBR is localized predominantly to the cytoplasm, indicating that the interaction of the LBR with chromatin is required for its retention in the inner nuclear membrane (Gajewski and Krohne, 1999).

The hydrophobic C-terminal half of the human LBR (hLBR) has remarkable structural similarities with the sterol reductase SR1 (Holmer et al., 1998) and it has been shown that the hLBR exhibits sterol C14 reductase activity (Silve et al., 1998). A mutation in the hLBR gene causes the autosomal recessive hydrops-ectopic calcification-'moth-eaten' (HEM)/Greenberg skeletal dysplasia. Cells carrying this mutation exhibit a dramatically reduced sterol C14 reductase activity (Waterham et al., 2003). Other mutations in the human (Hoffmann et al., 2002) and mouse (Shultz et al., 2003) LBRs cause an autosomal dominant disorder, the Pelger-Huet anomaly.

So far, only very limited information is available on the presence of LBR in invertebrate cells. The complete

sequencing of the *Drosophila* genome indicated that this model organism possesses a candidate gene to encode a LBR. Here, we describe the characterization and functional analysis of the protein encoded by the *Drosophila* gene CG17952 and demonstrate that it has some but not all properties in common with the vertebrate LBR.

Materials and Methods

Fly stocks, cell culture and transfections

D. melanogaster wild-type strain BERLIN was maintained on standard cornmeal-yeast-agar medium at 25°C. The following cell lines were used: S2 (*Drosophila* Schneider cell line 2; embryonic cell line of *Drosophila melanogaster*), Kc167 (hemocyte-derived cell line of *D. melanogaster*), COS-7 (kidney cells of *Cercopithecus aethiops*) and *Xenopus* A6 cells (kidney epithelial cells of *Xenopus laevis*). Vertebrate cell lines were cultured according to standard procedures (Lang and Krohne, 2003). S2 and Kc167 cells were cultivated in Schneider's *Drosophila* medium (Gibco/Invitrogen, Karlsruhe, Germany) containing 10% fetal calf serum (FCS), 50 U ml^{-1} penicillin, 50 U ml^{-1} streptomycin and 2 mM L-glutamine in the absence of CO_2. COS-7 and A6 cells were transfected using Rotifect (Roth, Karlsruhe, Germany), and S2 cells using Effectene (QIAGEN, Hilden, Germany).

Immunofluorescence

For immunofluorescence microscopy, embryonic or larval tissue was squashed between glass slides and coverslips and rapidly frozen on metal block cooled to –70°C. Coverslips were removed from the slides while frozen and the tissue was air dried for 10 minutes. Cells grown on coverslips or squashed tissues were fixed for 5 minutes in methanol at –20°C followed by a fixation for 1 minute with acetone at –20°C and transferred into PBS (137 mM NaCl, 3 mM KCl, 1.5 mM KH_2PO_4, 7 mM Na_2HPO_4, pH 7.4). Specimens were then processed for indirect immunofluorescence microscopy as described (Gajewski and Krohne, 1999; Lang and Krohne, 2003). Primary antibodies were diluted as follows: 1:1000 (polyclonal antibodies against the dLBR and lamin Dm0); 1:500 (polyclonal otefin antibodies); 1:10 (all monoclonal antibodies against *Drosophila* lamins and otefin); 1:300 (X223, polyclonal antibodies against *Xenopus* lamin B2) and as recommended by the supplier for commerically available antibodies against green fluorescent protein (GFP) and against nuclear pore proteins. Digitonin treatment of cultured cells was done as described (Gajewski and Krohne, 1999). Digital images were taken with a confocal laser scanning microscope (CLSM) (TCS SP, Leica, Heidelberg, Germany) and with a Zeiss Axiophot (Zeiss, Jena, Germany) equipped with a charge-coupled-device camera (using CamWare v1.00 software). Digital images taken by CLSM were recorded using standardized CLSM settings with a signal enhancement of 550-650 V. Signals in cells subjected to RNA interference (RNAi) and microinjected embryos were first visible at a signal enhancement of 760 V.

Microinjection of embryos and electron microscopy

D. melanogaster embryos (0 minutes to 30 minutes) were processed for microinjection using standard methods (1 μg dsRNA per μl H_2O) Microinjected and untreated embryos were analyzed at 24 hours, 48 hours and 72 hours after injection. Microinjected embryos were fixed, dehydrated and embedded for electron microscopy using standard techniques (Schoft et al., 2003). Ultrathin sections were analyzed with a Zeiss EM10 (Zeiss/LEO Oberkochen, Germany).

cDNA isolation, plasmids and sequence analysis

BLAST searches of Flybase (http://flybase.bio.indiana.edu/) were performed with the *X. laevis* LBR (accession number Y17842). The putative protein encoded by gene CG17952 exhibited the highest similarity with the *Xenopus* LBR (XLBR). The cDNA clones LD38760 and SD06601 of *D. melanogaster* gene CG17952 were generated by Celera Genetics and obtained from Invitrogen (Karlsruhe, Germany). The complete coding region of gene CG17952 was PCR amplified (using the primers 5′-TTTTCTAGAA-TGCAGCACTCGCCGAGCA-3′ and 5′-TTTGGATCCTTAGTA-GACCCTGGGCAGG-3′). The amplified DNA was cloned into the pCR®2.1-TOPO®-vector (Invitrogen, Karlsruhe, Germany) and pBluescript SK (Stratagene, Heidelberg, Germany). A DNA fragment encoding amino acids 16-334 of gene CG17952 was PCR amplified (using the primers 5′-AACTCGAGATGCAGGCACGTTTTTT-CGACCGCAATT-3′ and 5′-TTGGTACCCTGGCCGTACAGC-TCCATGTC-3′), and cloned into pEGFP-N1 (CLONTECH Laboratories, Heidelberg, Germany). A DNA fragment encoding amino acids 17-262 of gene CG17952 was PCR amplified (using the primers 5′-AACATATGCGTTTTTTCGACCGCAATTCATACAC-3′ and 5′-AACTCGAGACGCTTGGAGGACTTCTCATCGTCG-3′) and cloned into pET21a (Novagene, Merck, Darmstadt, Germany). PCR and sequence analysis have been described (Lang et al., 1999). For the prediction of protein secondary structure, we used the program PIX (http://www.hgmp.mrc.ac.uk/Registered/Webapp/pix/8).

dsRNA production and conditions for RNAi in *Drosophila* cell culture

Double-stranded RNA (dsRNA) production and RNAi in *Drosophila* S2 Schneider cells were performed as described (Clemens et al., 2000). Distinct DNA fragments approximately 800-900 bp long containing the coding sequences of the *Drosophila* genes CG17952 and lamin Dm0 were amplified by PCR. Each primer used for amplification contained the T7 RNA polymerase binding site (GAATTAATAC-GACTCACTATAGGGAGA) followed by the specific sequences (CG17952: 5′-CCCAGTCCAAGCAGCCCAGCC-3′ and 5′-GCAA-AGGCACCCACCACTCGT-3′; lamin Dm0: 5′-ATGTCGAGCAA-ATCCCGACGT-3′ and 5′-GCGACTGCTTCAACTTGGCATC-3′). PCR products were cloned into the pCR®2.1-TOPO®-vector (Invitrogen, Karlsruhe, Germany), DNA fragments were then isolated by digestion with *Eco*RI, purified by using the Gel Extraction Kit (Qiagen, Hilden, Germany) and transcribed with the MEGASCRIPT T7 transcription kit (Ambion, Huntingdon, UK). The RNA was annealed in 20 μl H_2O as recommended and stored at –70°C.

S2 cells were diluted to a final concentration of 5×10^5-7.5×10^5 cells ml^{-1} in Schneider's *Drosophila* medium (Gibco/Invitrogen, Karlsruhe, Germany) without fetal calf serum and antibiotics. Cells were plated and transfected with the dsRNA as described (Clemens et al., 2000). After an incubation period of 24 hours, the cells were harvested, split and transferred to a 24-well cell culture dish (Greiner BIO-ONE, Frickenhausen) containing one coverslip of 12 mm diameter in each well. The cells were incubated for additional 12 hours, 24 hours, 36 hours, 48 hours, 52 hours and 66 hours, and analyzed.

Expression and purification of bacterially expressed *Drosophila* lamin Dm0

The complete coding sequences of *Drosophila* lamin Dm0 had been cloned into the pET17 vector (Stuurman et al., 1996) and expressed in the bacterial strain *Escherichia coli* BL21 Codon-Plus (Stratagene, Heidelberg, Germany). Lamin Dm0 was extracted from bacterial inclusion bodies and purified by ion exchange chromatography as described (Gieffers and Krohne, 1991). Anion exchange chromatography was performed in 8.5 M urea at pH 7.5 [8.5 M urea, 20 mM Tris-HCl pH 7.5, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonylfluoride (PMSF)] and cation exchange chromatography in 8.5 M urea at pH 5.8 (8.5 M urea, 50 mM sodium acetate pH 5.8, 1 mM dithiothreitol, 0.2 mM PMSF).

Antibodies

Polyclonal antibodies were generated in guinea pigs and BALB/c mice as described (Cordes et al., 1991). As antigens, we used the full-length protein (lamin Dm0) or peptides equivalent to amino acids 17-262 of CG17952 or amino acids 2-264 of otefin. Peptides of CG17952 and otefin were expressed in *E. coli* BL21 Codon-Plus and affinity purified as described (Schoft et al., 2003).

Mouse monoclonal antibodies have been described that are specific for *Drosophila* lamin Dm0 (ADL67, ADL195, ADL85) (Klapper et al., 1997; Krohne et al., 1998), *Drosophila* lamin C (LC28) (Klapper et al., 1997; Krohne et al., 1998), otefin (Ashery-Padan et al., 1997) and *Xenopus* lamin B2 (X223) (Lourim and Krohne, 1993). Mouse monoclonal antibodies against nuclear pore complex proteins (mab414; Berkeley Antibody Company), α-tubulin (Sigma T-5168; Sigma, Deisenhofen, Germany) and GFP (Roche Diagnostics, Mannheim, Germany) were commercially available. Polyclonal CG17952 (dLBR) antibodies of two guinea pig antisera were affinity purified using bacterially expressed CG17952 (amino acids 17-262) coupled to CNBr-activated Sepharose™ 4B (Amersham Pharmacia, Freiburg, Germany).

SDS-PAGE and immunoblotting

Sodium-dodecyl-sulphate polyacrylamide-gel electrophoresis (SDS-PAGE) and immunoblotting was performed as described (Lang and Krohne, 2003). To allow the direct comparison of RNAi-treated and control cells, total protein of 1.25×10^5 S2 cells were separated per lane on the same SDS-PAGE and processed for immunoblotting.

To detect [^{35}S]methionine labeled proteins after separation by SDS-PAGE, gels were incubated for 1 hour in dimethylsulfoxide, followed by an incubation for 3 hours in Rotifluoreszint D (Roth, Karlsruhe, Germany) and finally for 1 hour in H_2O. Gels were dried and exposed to X-ray films. Densitometric analysis of X-ray films was performed using Scion Image for Windows (Scion Corporation, Maryland, USA).

Expression in *S. cerevisiae*

The CG17952 cDNA was amplified (primers: 5′-TTTGAATTA-ATGCAGCACTCGCCGAGCA-3′ and 5′-TTTCTCGAGTTAGTAG-ACCCTGGGCAGG-3′), subcloned into the pCR®2.1-TOPO® vector, digested with *Eco*RI and cloned into the yeast-expression vector pYX212. Plasmids ERG24-pYX212, FACKEL-pYX212, control-pYX212 and the *S. cerevisiae* mutant strain erg24::*LEU2 his3Δ200 leu2Δ1 trp1Δ63 ade2-101 lys2-801 ura3-52* have been described (Schrick et al., 2000). Yeast transformation was performed as in Gietz et al. (Gietz et al., 1992). Permissive growth conditions were in –URA + 20 mM $CaCl_2$ synthetic medium. Cells were grown in calcium-poor yeast extract-peptone-dextrose medium plus 0.01% adenine (YPAD) medium to assay growth under restrictive conditions.

Sterol extraction and mass spectrometry

S. cerevisiae cells were grown in YPAD medium for 24 hours. Sterols were extracted as follows. Cells were separated from the medium by centrifugation at 4°C at 2000 ***g*** for 5 minutes, washed twice with buffer (0.1 M potassium phosphate pH 7.4, 1% ethanol v/v) and once with 0.1 M potassium phosphate (pH 7.4). The pellet was resuspended in methanol containing 40% KOH (w/v). Saponification was carried out by refluxing in a Liebig condenser at 90°C for 1 hour. After cooling to room temperature, sterols were extracted with n-hexane. The organic phase was transferred to an evaporation flask and concentrated to 2-3 ml. Sterols were analyzed by mass spectrometry (GC 8060, Fisons Instruments MD800, Thermo Finnigen, France) using a DB5 MS column (length 30 meters, diameter 250 μm) and helium as the carrier gas, which flowed with a constant pressure of 90 kPa through the column. The column oven temperature was programmed at 60-300°C with a temperature increase of 5°C per minute. Detection was obtained using a flame ionization detector with a detector oven temperature of 300°C.

In vitro translation

Proteins CG17952 and lamin Dmo contained in the pET17, pET21a or pBluescript SK vector were in vitro synthesized by coupled transcription and translation using the TNT system (Promega, Madison, WI, USA) as recommended by the supplier. For radioactive labeling of proteins during synthesis, 0.5 μg of plasmid DNA and 40 μCi [^{35}S]-methionine (Amersham) were used per experiment.

In vitro binding assays

For in vitro binding studies, bacterially expressed and purified lamin Dm0 or the peptide equivalent to amino acids 17-262 of CG17952 were solubilized in PBS containing 2 M urea (CG17952) or 4 M urea (lamin Dm0) at a final concentration of 1 mg ml^{-1}. Of this stock solution, 0.5 μl was mixed with 19.5 μl PBS. Wells of a 96-well plate (Greiner BIO-ONE) were coated with the desired protein (0.5 μg protein in 20 μl PBS per well) by incubation for 2 hours at 18°C, followed by an incubation with PBS containing 0.5% bovine serum albumin (PBS/BSA) for 2 hours at 18°C. Wells were washed three times with PBS and then incubated with the in vitro synthesized protein (3 μl in vitro translated protein with 17 μl PBS/BSA per well) for 2 hours at 18°C. Wells were finally washed three times with PBS and bound proteins were solubilized in sample buffer for SDS-PAGE. Control wells were coated with BSA. As a further control, the in vitro translated protein was preincubated for 1 hour at 18°C with the protein that had been used to coat the wells (3 μl in vitro translated protein, 17 μl PBS/BSA, 1 μg recombinant protein) and then added to the coated well. All other steps were as described above.

Immunoprecipitations and cell fractionation

All immunoprecipitation steps were performed at 4°C using the 13,000 ***g*** supernatants of *Drosophila* S2 and Kc167 cells that had been extracted with immunoprecipitation buffer (1% Triton X-100, 150 mM NaCl, 20 mM Tris, 1mM PMSF, 0.1 mg ml^{-1} trypsin inhibitor, pH 7.4) as described (Lang and Krohne, 2003). The 13,000 ***g*** supernatant was incubated with polyclonal guinea pig or mouse antibodies against CG17952 coupled to protein-A/Sepharose (Pharmacia Biotech, Uppsala, Sweden) exactly as described (Lang and Krohne, 2003). Reticulocyte lysates containing co-translated [^{35}S]-methionine-labeled lamin Dm0 and CG17952 (amino acids 17-262) were used for immunoprecipitation with CG17952 antibodies. Urea extractions of S2 cells or transfected COS-7 were performed as described (Schoft et al., 2003).

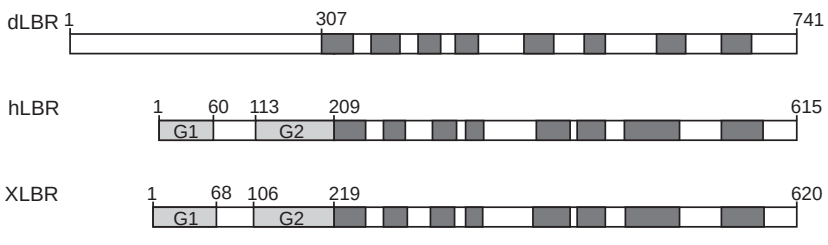
In vitro chromatin-binding assay

The preparation of *Xenopus* sperm chromatin and the heat-treated 200,000 ***g*** supernatant (S_{200}) of unfertilized *Xenopus* eggs has been described (Gajewski and Krohne, 1999). For chromatin binding, the bacterially expressed and purified N-terminal domains of the XLBR (Gajewski and Krohne, 1999) and CG1792 were precipitated with methanol/chloroform (Schmidt et al., 1994), and resuspended at a concentration of 1-2 μg μl^{-1} in H_20. The *Xenopus* and *Drosophila* proteins were added to heat-treated S_{200} (0.5 μg XLBR per 20 μl extract or 0.5 μg CG17952 per 20 μl extract) and incubated for 5 minutes at 4°C. Subsequently, insoluble components were pelleted. The supernatants were incubated in the presence or absence of sperm chromatin (20,000 sperm per μl S_{200}) for 90 minutes at room temperature. Following incubation, the extracts were processed as described (Gajewski and Krohne, 1999). Proportional amounts of each sample were prepared for SDS-PAGE and immunoblot analysis.

A

```
hLBR    ----MPSRKFADGEVVRGRWPGSSLYYEVEILS----HDSTSQLYTVKYKDGTELELKEN  52
XLBR    MIKKMPGQKYEIGETVMGRWPGSSLYYEVQVVG----FNSKSQEYKVLYKDGTDLDLKEG  56
dLBR    -MQHSPSTTTDHIHFAARFFDRNSYTMDRRLRRPRRTEDVSSGPLLAQSKQPTLLPVTRR  59
             *                *                  *       *  * *

hLBR    DIKPLTSFRQRKGGSTSSSPSRRRG-----------------SRSRSRSRSPGRPPKSA  94
XLBR    DIKRQNIFNRKRSSSPSRRRSRSRSPG-------------RSRSPARRRSPLRPSSPG 103
dLBR    TGSVTAAAATATATATAGPATRTRASPSPRNKVVAPPSPDLGPRTRRSSRPRSSVGPLTGS 119
                          * *                  *   * **   *

hLBR    RRSASASHQADIKEARR--EVEVKLTPLILKPFGN----SISRYNGEP------------ 136
XLBR    RPAKNGHQSSLIRDIKKGDTLQVHLTPVKLQDY------STGKHNGEP------------ 145
dLBR    GSGSSLPIKAAIKARTPIPEVSEVSSPIRLSTSNLPMTLTTNTSSGAPNKAFNTSSVNSG 179
                  *             *  *              * *

hLBR    EHIERNDAPHKNT----QEKFSLSQ-ESSYIATQYSLRPRREEVKLKEIDSKEEKYVAKE 191
XLBR    EGFEKITTRHRATPVKAIELMEEEL-ERNEKVLHYSLSPRQESSIPTGIVLADS--VPTE 202
dLBR    NSFSRTTTSSTTTTTERIEIRAEGDGEVDTDSIRKRITERLRRSVSKTISNLAGTPVTNT 239
                    *     *     *           *     *     *

hLBR    LAVRTFEVTPIRAKD------------------------------------------- 206
XLBR    TLPEMTEKNAENPK-------------------------------------------- 216
dLBR    EEGSRYSRSVSRSVYDDEKSSKRSYSTGEEDIDEEDELEEDQFRSFNVTRKSATPAEISC 299

hLBR    ------LEFGGVPGVFLIMFGLPVFLFLLLLMCKQKDPSLLNFPPP-LPALYELWETRVF 259
XLBR    ------LAFGGAIGCFLFMVCVPALLYYLLVVCGQQYTS--GYP------FVELLDIQVF 262
dLBR    RQLKAPREFGGWLGAFLLLLLLPTAVYYLTWSCTARNACQFKHLNLGILLDVNYLTRQVF 359
              ***  * **     *     *   *                        **

hLBR    GVYLLWFLIQVLFYLLPIGKVVEGTPLIDGRRLKYRLNGFYAFILTSAVIG-TSLFQGVE 318
XLBR    GFFVLWTLLQVILYLLPLGKVVDGAQLKNGKRLKYRISGCSAFFLTAAIMAGMKYYYEIN 322
dLBR    QPRVVGAFAAYQVVVFLLVALLPGRRVHLTR-ETYKFNCLAVSLTLLIAGGVAEYLKYPV 418
                              *       *

hLBR    FHYVYSHFLQFALAATVFCVVLSVYLYMRSLKAPRNDLSPAS------SGNAVYDFFIGR 372
XLBR    FLYIFEHYLQFAASATLFSFLLSIYLYVRSYKVPNEELSWAAN-----SGNFIYKFVMGR 377
dLBR    VTFVLRHYLRFCIFGLVGAFVAAAWSYWLVDTAKYNVLRQTLTNDYGRTGSFVVDFALGR 478
          * * *               *         *           *     *  **

hLBR    ELNPR-IGTFDLKYFCELRPGLIGWVVINLVMLLAEMKIQDRAVP--------------- 416
XLBR    EINPR-IGNLDLKVFVVIRQALMSWVLINLIMLFAEMKVHKWDEP--------------- 421
dLBR    QLNPKWLGRVDWKQFQYRLSLVTTLIYATCYIYQTLVWPQKPQLGEQEGYLYQAKYYWNN 538
          **   *  * * *

hLBR    ---SLAMILVNSFQLLYVVDALWNEEALLTTMDIIHDGFGFMLAFGDLVWVPFIYSFQAF 473
XLBR    ---SLSMILVNSFQLLYVLDGFWNEEYFLMSPDIVRDGFGFLLAFGSLAVAPFTYSLQTY 478
dLBR    VNYDPATLFSASCLLFYVLDAIIFEHHLSSSFELQHEGYGCLLLRYAATPYLLTAVTKY 598
                  *  * ** *   *           * *  *

hLBR    YLVSHPNEVSWPMASLIIVLKLCGYVIFRGANSQKNAFRKNPSDPKLAHLKTIHTSTGKN 533
XLBR    YLVNNPVDLSRQAASAIVALKFLGYIIYRGANNQKCAFRQNPDDPRLSHLKTIPTSAGSK 538
dLBR    FYEQRVPISCWYAPLAVAALLSLGLLVKRFSCAYKYKYRLNSQSPIFANIETIHTYQGSR 658
                        *   *    *    *   * *   *      ** *  *

hLBR    LLVSGWWGFVRHPNYLGDLIMALAWSLPCGFNHILP-YFYIIYFTMLLVHREARDEYHCK 592
XLBR    LLISGWWGFVRHPNYLGDIIMALAWCLACGFNHILP-YFYVIFLTLLLIDRAARDEQRCR 597
dLBR    LLLSGMWGWVRQPNYLGDIVALLALAAPMALRPAWPPVLGLSLIILLLLHRATRANARNQ 718
        ** ** ** ** ******    **          *         **  *  *

hLBR    KKYGVAWEKYCQRVPYRIFPYIY 615
XLBR    EKYGLDWDKYCQHVRYRLLPYVY 620
dLBR    ARYHSSWQRYSTQVRSYILPRVY 741
          *   *  *    *  *
```

B



Results

Characterization of dLBR, the *D. melanogaster* protein encoded by gene CG17952

By BLAST searches of the *Drosophila* Flybase, we noted that the putative protein encoded by *Drosophila* gene CG17952 exhibited the highest similarity with the *X. laevis* LBR. CG17952 has been mapped to the chromosomal region 57F10-11. The complete sequencing of the expressed-sequence-tag clones LD38760 and SD06601 (accession number AJ606680) revealed some minor differences from the genomic sequence published by Celera Genetics. We could confirm the sequences of the two expressed-sequence-tag clones by sequencing the corresponding PCR amplified genomic DNA fragments. The cDNAs derived from gene CG17952 encode a protein of 741 amino acids (Fig. 1, dLBR) with a calculated molecular mass of 82,948 Da and a p*I* of 9.83. Based on the results described in this report, we named this protein encoded by CG17952 *Drosophila* lamin B receptor (dLBR).

Secondary structure analysis of dLBR (Fig. 1A,B) predicted eight putative transmembrane domains in the C-terminal half of the molecule. Transmembrane segments 1-6 are similar in length and position to the transmembrane domains 1-6 of hLBR and XLBR (Fig. 1A,B), whereas the putative membrane spanning domains 7 and 8 of the *Drosophila* protein are markedly shorter than those of the vertebrate LBRs. Secondary structure prediction revealed that amino acids 1-307 of the dLBR are not organized in two globular domains, G1 and G2 (Fig. 1B), that are characteristic for the vertebrate LBRs (Ye et al., 1997). The *Drosophila* protein and both vertebrate LBRs exhibit the highest degree of identity in the hydrophobic C-terminal region that contain the eight transmembrane domains (22.8% identity for amino acids 308-

Fig. 1. Amino acid sequence and scheme of the *D. melanogaster* lamin B receptor (dLBR) encoded by gene CG17952. (A) Amino acid sequence comparison of the lamin B receptor of human (hLBR), *Xenopus laevis* (XLBR) and dLBR. The membrane-spanning domains are underlined. The sequence used for the generation of polyclonal antibodies against the *Drosophila* protein is marked by a dotted line. Amino acids identical in all three species are printed in bold and marked by asterisks. Amino acids that are identical in the two vertebrates are also printed in bold. (B) Schematic drawings of the dLBR, hLBR and XLBR. The positions of individual amino acids are marked by numbers. Boxes depict transmembrane segments (black) and two globular domains (G1, G2; gray) in the N-terminal half of the human and *Xenopus* LBR; these globular domains are not predicted for the dLBR sequence. The nucleotide sequence of the *D. melanogaster* gene CG17952 (dLBR) is available under accession number CG17952 of the Berkeley *Drosophila* Genome Project (BDGP) Database. The corrected sequence of the dLBR shown here is available under accession number AJ606680.

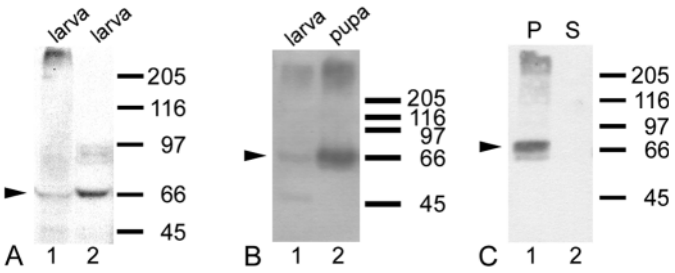


Fig. 2. Characterization of the dLBR protein. (A-C) Total larval proteins of *Drosophila melanogaster* second (A, lane 1) and third (A, lane 2; B, lane 1) instar, and pupa (B, lane 2) were separated by SDS-PAGE and immunoblotted with affinity-purified dLBR antibodies (A) or non-purified dLBR antibodies (B,C). In each lane, total proteins of one animal were loaded. (C) Aliquots of S2 cells were incubated with 8 M urea and fractionated by 100,000 ***g*** centrifugation into a supernatant (S) and a pellet containing predominantly membranes (P). Proportional amounts of proteins of both fractions were separated by SDS-PAGE and immunoblotted with dLBR antibodies. A polypeptide of M_r 66,000 is detected by the dLBR antibodies (A-C, arrowheads). dLBR does form aggregates in SDS sample buffer resulting in a smear close to the top of the gel (A-C; Fig. 7A, Fig. 8A). Molecular masses of reference proteins (in kDa) are marked.

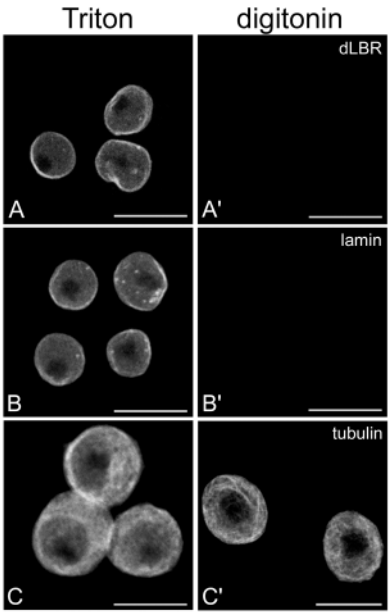


Fig. 3. The dLBR is localized to the inner nuclear membrane. Indirect immunofluorescence microscopy (A-C; A′-C′) of S2 cells after staining with antibodies against the dLBR (A,A′), lamin Dm0 (B,B′) and α-tubulin (C,C′). Cells were fixed with formaldehyde and then permeabilized with Triton X-100 (A-C) or digitonin (A′-C′). The inner nuclear membrane and nuclear interior are only accessible to antibodies in Triton-treated cells. Scale bars, 10 μm.

741 of dLBR; 66.7% identity for amino acids 650-676 of the dLBR). By contrast, the similarity of the amino acid sequences between the vertebrate LBRs and the *Drosophila* protein is marginal in the N-terminal region (7.8% identity in amino acids 1-307 of the dLBR). By comparison, the two vertebrate LBRs, hLBR and XLBR, demonstrate a high sequence identity in the C-terminal (57.8% identity in amino acids 220-620 of XLBR) as well as in the N-terminal region (36.5% identity in amino acids 1-219 of XLBR). Despite the low sequence identity, the N-terminal domains of both dLBR and the vertebrate LBRs are very basic (hLBR p*I* = 8.85, dLBR p*I* = 10.34) and rich in hydroxy amino acids (hLBR, 18.7%; dLBR, 29.0%), and possess several putative phosphorylation sites for different kinases.

To enable the biochemical and immunocytological analysis of the dLBR protein, we generated antibodies against its N-terminal domain (Fig. 1). When total proteins from *Drosophila* larvae, pupae (Fig. 2A,B), and Schneider S2 cells were analyzed by immunoblotting with affinity-purified dLBR antibodies, one polypeptide band with an apparent relative molecular weight of 66,000 was detected. In addition, we often detected a smear close to the top of the gel (see, for example, Fig. 2A-C, lane 1), indicating that the dLBR forms aggregates that cannot easily be dissociated in sample buffer for SDS-PAGE. Aggregates were also observed when the sample was not heated, when reducing agents were omitted and when urea was included.

The extraction of Schneider S2 cells with 8 M buffered urea and the fractionation of this cell extract by high-speed centrifugation into a membrane pellet (Fig. 2C, lane 1) and a supernatant (Fig. 2C, lane 2) revealed that the M_r 66,000 polypeptide remains in the pellet fraction. This behavior is a characteristic of integral membrane proteins. The experimental data agree with the structural predictions obtained by computer analysis (Fig. 1).

Immunofluorescence microscopy revealed that the dLBR is, in somatic cells, predominantly localized to the inner nuclear membrane. This localization can be concluded from the comparative analysis of formaldehyde-fixed Schneider S2 cells after extraction with Triton X-100 (Fig. 3A-C) or the selective permeabilization of the plasma membrane with digitonin (Fig. 3A′-C′). When Triton-permeabilized cells were incubated with antibodies against the dLBR, a staining of the nuclear envelope was observed (Fig. 3A), whereas the dLBR was not accessible to antibodies in digitonin-treated cells (Fig. 3A′). Control experiments performed in parallel with antibodies against a protein localized on the nucleoplasmic side of the inner nuclear membrane (lamin Dm0; Fig. 3B,B′) and a cytoplasmic protein (α-tubulin: Fig. 3C,C′) revealed that the digitonin treatment had permeabilized the plasma membrane (Fig. 3C′) but not the nuclear membrane (Fig. 3A′,B′), and that antigens localized in the nuclear interior were only accessible in Triton-extracted cells (Fig. 3A,B).

To determine whether the *Drosophila* LBR has additional properties in common with the vertebrate LBRs, we generated a plasmid vector that allowed us to express a dLBR-GFP fusion protein. This chimeric protein possessed most of the N-terminal domain and the first transmembrane segment of the dLBR (amino acids 16-334 of the dLBR) followed by GFP. For the hLBR, it has been shown that this part of the molecule is sufficient for its targeting to the inner nuclear membrane in transfected cells (Smith and Blobel, 1993; Soullam and Worman, 1993; Ellenberg et al., 1997). In transfected *Xenopus* A6 cells (Fig. 4A,B), COS-7 cells (Fig. 4C) and *Drosophila* S2 cells (Fig. 4D), this dLBR-GFP fusion protein always localizes to the nuclear envelope of transfected cells and co-localized

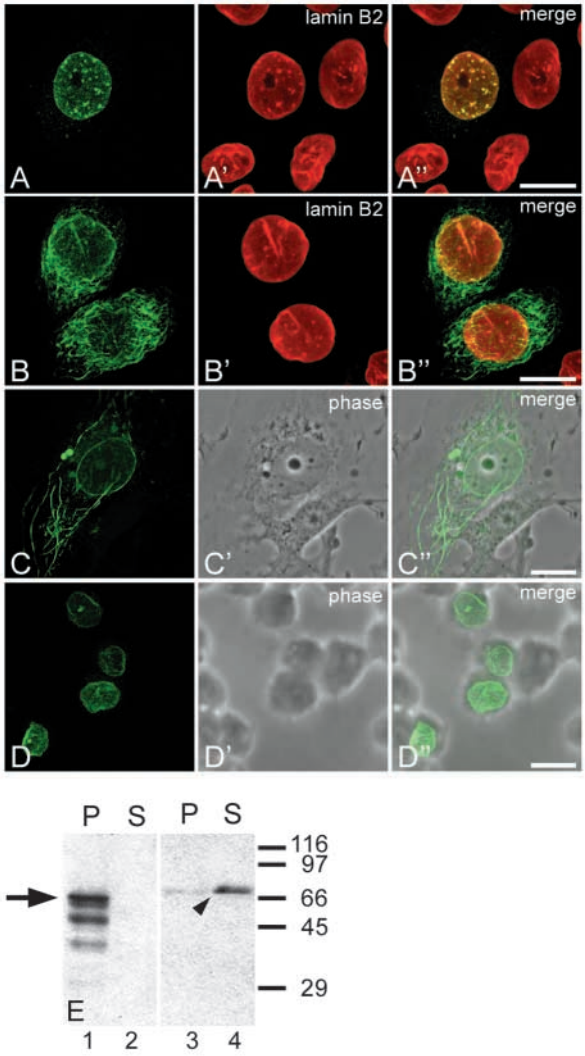


Fig. 4. The dLBR is targeted to the nuclear membrane of vertebrate and insect cells. (A) Expression of amino acids 16-334 of the dLBR as a GFP fusion protein (dLBR-GFP) in *Xenopus* A6 cells (A,B), COS-7 cells (C) and *Drosophila* S2 cells (D). This fusion protein comprises the N-terminal domain and the first membrane-spanning segment. The fluorescence of the GFP fusion protein in the transfected cells is shown (A-D) with the staining of endogenous lamin B2 by indirect immunofluorescence microscopy with antibody X223 (A′,B′) or a phase-contrast image (C′,D′,A″-D″; merge – overlays). Digital images taken by CLSM are shown. Scale bars, 10 μm. (E). Biochemical properties of the fusion protein dLBR-GFP in COS-7 cells. Aliquots of transfected COS-7 cells were incubated with buffered 8 M urea and fractionated by 100,000 ***g*** centrifugation into a supernatant (S) and a pellet fraction (P). Proteins were separated by SDS-PAGE and immunoblotted with antibodies against GFP (lanes 1, 2) or lamin B2 (lanes 3, 4). The position of dLBR-GFP is marked by an arrow and lamin B2 is marked by an arrowhead. The two polypeptide bands with higher mobility than the dLBR-GFP that were reacting with the GFP antibodies (lane 1) represent degradation products. Molecular masses of reference proteins (in kDa) are marked.

with lamins (Fig. 4A-B″). In cells over-expressing dLBR-GFP, the fusion protein was also localized to the endoplasmic reticulum (Fig. 4B,C). When transfected COS-7 cells were extracted with 8 M urea and fractionated as described above for Schneider S2 cells, the dLBR-GFP was recovered in the membrane pellet, demonstrating that the first membrane-spanning domain of the dLBR is localized between amino acids 308 and 332 (Fig. 4E, lanes 1 and 2). The membrane-associated lamins were recovered in the urea supernatant (Fig. 4E, lanes 3 and 4).

Identification of cellular components interacting with the dLBR

Our data indicate that the dLBR is directly comparable to the vertebrate LBRs in its topological organization in the inner nuclear membrane. The N-terminal 307 amino acids of the dLBR and the N-terminal domain of the vertebrate LBRs are both localized to the nucleoplasmic side of the inner nuclear membrane. Proteins in this subdomain of the nuclear envelope are close to lamins and chromatin. Therefore, we tested whether this domain of the dLBR binds to the lamina/lamins and/or chromatin.

To test the interactions of lamins with dLBR, we used two *Drosophila* cell lines (S2 and Kc167 cells). S2 and Kc167 cells differ in their expression of lamins. Kc167 cells express lamins Dm0 and C, whereas S2 cells express lamin Dm0 in all cells but lamin C in fewer than 1% of the cells. Cell extracts were prepared for immunoprecipitation in nearly physiological buffers in the presence of 1% Triton X-100. Following centrifugation of these extracts at 13,000 ***g***, at least 50% of the dLBR and of lamins could be recovered in the supernatant (data not shown) (see Gajewski and Krohne, 1999; Lang and Krohne, 2003). Sucrose gradient centrifugation (5-30% sucrose) of the 13,000 ***g*** supernatant revealed that solubilized dLBR was present in all fractions, whereas the vast majority of lamin Dm0 possessed sedimentation coefficients of 6.5 S and smaller (data not shown). To verify whether a subpopulation of the dLBR and lamins was present in a common complex, we performed immunoprecipitations with mouse and guinea pig polyclonal antibodies against dLBR. Using the extract of Kc167 cells we consistently detected co-immunoprecipitated lamin Dm0 (Fig. 5A, lanes 1 and 2) but no lamin C (Fig. 5A, lane 3), indicating that the cell extracts contained solubilized protein complexes comprising the dLBR as well as lamin Dm0. In addition, we immunoprecipitated the dLBR from reticulocyte lysates that contained the in vitro translated N-terminal domain of the dLBR (amino acids 17-262) as well as the full-length in vitro translated lamin Dm0 (Fig. 5B, lane 1). When we used our polyclonal dLBR antibodies for immunoprecipitation, we detected the N-terminal domain of the dLBR as well as lamin Dm0 in the immunoprecipitate (Fig. 5B, lane 3).

We wanted to use a method independent of antibodies to assess the binding of the N-terminal domain of the dLBR to lamin Dm0, so we coated enzyme-linked immunosorbent assay (ELISA) plates with dLBR fragments consisting of amino acids 17-262 and tested the binding of in vitro translated lamin Dm0 (Fig. 5C). Our results demonstrate that the N-terminal domain of the dLBR is sufficient for the binding to lamin Dm0 in a solid phase assay (Fig. 5C, lanes 1 and 2). The specificity of the interaction is shown by the preincubation of the in vitro translated lamin Dm0 with the N-terminal domain of the dLBR. The preincubated lamin Dm0 did not show a significant binding to the ELISA plate coated with the dLBR (Fig. 5C, lane 3; Table 1). We also confirmed the

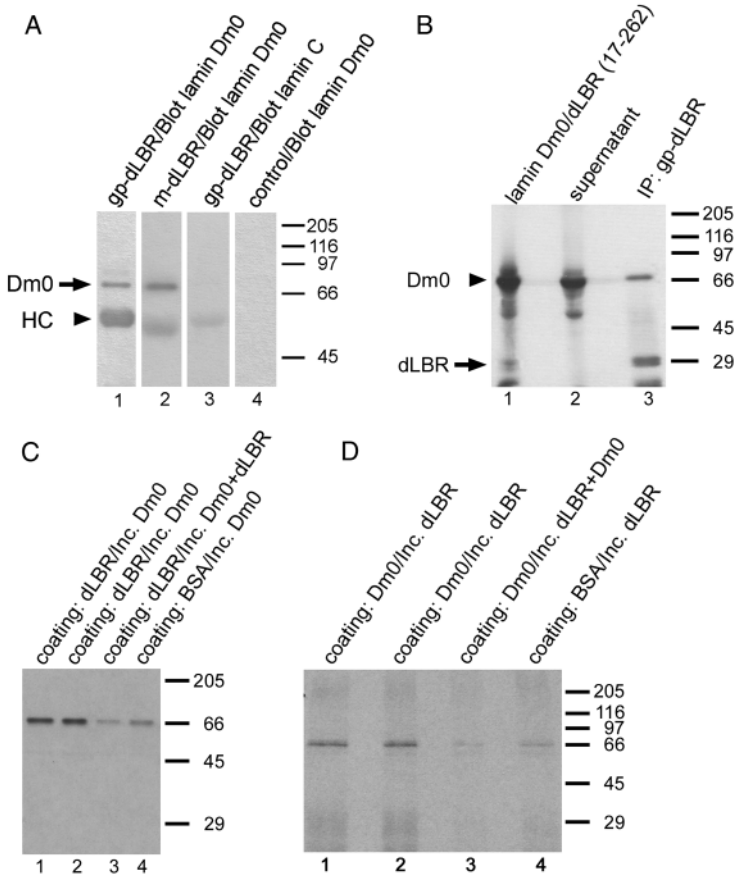


Fig. 5. (A) Co-immunoprecipitation of the dLBR and lamins from the 13,000 ***g*** supernatant of *Drosophila* Kc167-cells extracted with immunoprecipitation buffer. Immunoprecipitations were performed with polyclonal guinea pig (gp-dLBR; lanes 1, 3) and mouse (m-dLBR; lane 2) antibodies against dLBR that were bound to protein-A/Sepharose. As a control (control; lane 4), proteins of the extract bound to the protein-A/Sepharose in the absence of antibodies were analyzed. Proteins of immunoprecipitates were separated by SDS-PAGE and immunoblotted with mouse monoclonal antibodies against lamin Dm0 (lanes 1, 4; Blot lamin Dm0) and lamin C (lane 3; Blot lamin C), and with guinea pig antibodies against lamin Dm0 (lane 2; Blot lamin Dm0). The position of lamin Dm0 is marked by an arrow (Dm0) and the heavy chains (HC) of the antibodies by an arrowhead. (B) Co-immunoprecipitation of [^{35}S]-methionine-labeled dLBR (amino acids 17-262) and lamin Dm0 from reticulocyte lysates with guinea pig antibodies against dLBR (gp-dLBR) that were bound to protein-A/Sepharose. Both proteins had been translated in the reticulocyte lysate [lane 1; lamin Dm0/dLBR (17-262)]. Total proteins of the reticulocyte lysate (lane 1), proteins remaining in the supernatant after immunoprecipitation (lane 2, supernatant) and immunoprecipitated proteins (lane 3; IP, gp-dLBR) were separated by SDS-PAGE and visualized by fluorography. The positions of the dLBR (arrow) and lamin Dm0 (arrowhead) are marked. (C,D) In vitro binding of [^{35}S]-methionine-labeled lamin Dm0 to the immobilized N-terminal domain of the dLBR (C; amino acids 17-262 of the dLBR) and of [^{35}S]-methionine-labeled dLBR to the immobilized lamin Dm0 (D). Wells of ELISA plates that had been coated with the dLBR (C; lanes 1-3; coating, dLBR), lamin Dm0 (D, lanes 1-3) or BSA (lanes 4 in C,D; coating, BSA) were incubated with [^{35}S]-methionine-labeled lamin Dm0 (C; lanes 1, 2, 4; Inc. Dm0) or with [^{35}S]-methionine-labeled dLBR (D; lanes 1, 2, 4; Inc. dLBR). As controls [^{35}S]-methionine-labeled lamin Dm0 was preincubated with the dLBR in solution (C; lane 3; Inc. Dm0 + dLBR) or [^{35}S]-methionine-labeled dLBR was preincubated with lamin Dm0 in solution (D; lane 3; Inc. dLBR + Dm0) and then added to the wells. Proteins bound to the wells were separated by SDS-PAGE. X-ray films of both gels are shown. Quantification of the bound radioactively labeled proteins are shown in Tables 1 and 2. Molecular masses of reference proteins (in kDa) are marked in A-D.

in vitro binding of the dLBR to lamin Dm0 by using ELISA plates coated with purified lamin Dm0 that were incubated with the full-length in vitro translated dLBR (Fig. 5D). The in vitro translation of the full-length dLBR was always less efficient that of the lamin Dm0, so the signals seen on the X-ray film were weaker (Tables 1, 2). Experiments with in vitro translated *Drosophila* lamin C could not be performed because this lamin had already formed polymers in the reticulocyte lysate.

A further characteristic feature of the N-terminal domain of the vertebrate LBR is its binding to chromatin. To elucidate the chromatin binding properties of the *Drosophila* LBR, we incubated the dLBR peptide (amino acids 17-262 of the dLBR) with demembranated *Xenopus* sperm in a fractionated *Xenopus* egg extract. As a control, we performed the experiment with the N-terminal domain of the XLBR, a protein known to bind to sperm chromatin (Gajewski and Krohne, 1999). Our data (Fig. 6A) demonstrate that the soluble N-terminal domain of the dLBR does bind to sperm chromatin and could be recovered together with the sperm in the pellet fraction (Fig. 6A, lane 4) but the dLBR could not be pelleted in the absence of sperm chromatin. Identical results were obtained with the N-terminal domain of the XLBR (Fig. 6B). Our controls (Fig. 6A,B, lanes 5) demonstrate that proteins of the sperm chromatin do not cross-react with the antibodies used.

Table 1. Quantification of the bound [35S] methionine-labeled lamin Dm0 shown in lanes 1-4 of Fig. 5C

	Background	Lane 1	Lane 2	Lane 3	Lane 4
Total pixels	19.07	62.01	64.25	29.04	36.98
Pixels above background	0	42.94	45.18	9.97	17.91

These values derive from the densitometric analysis of the X-ray films shown in Fig 5C using the program Scion Image for Windows (http://www.scioncorp.com).

Table 2. Quantification of the bound [^{35}S]-methionine-labeled dLBR shown in lanes 1-4 of Fig. 5D

	Background	Lane 1	Lane 2	Lane 3	Lane 4
Total pixels	55.63	78.28	79.30	60.18	67.46
Pixels above background	0	22.65	23.67	4.55	11.83

These values derive from the densitometric analysis of the X-ray films shown in Fig 5D using the program Scion Image for Windows.

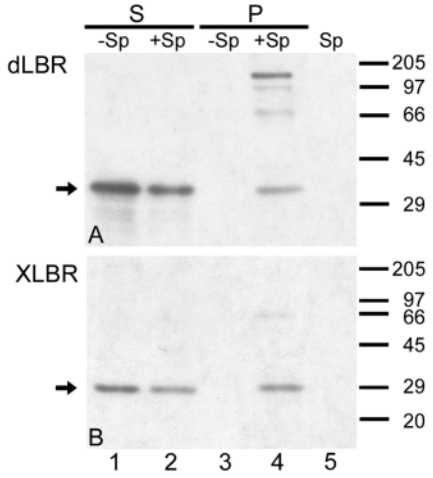


Fig. 6. In vitro binding of the bacterially expressed N-terminal domain of *Drosophila* LBR (amino acids 17-262 of the dLBR) and *Xenopus* LBR (amino acids 4-210 of the XLBR) to sperm chromatin. Soluble proteins of heat-treated *Xenopus* egg extract (S_{200}) supplemented with dLBR (A) or XLBR (B) were incubated in the presence (+Sp) (lanes 2, 4) or absence (–Sp) (lanes 1, 3) of demembranated sperm chromatin, then fractionated into supernatants (S) and pellets (P) by centrifugation. Proteins of each fraction and of sperm chromatin that had not been incubated (SP; lane 5) were separated by SDS-PAGE and analyzed by immunoblotting with antibodies against the dLBR (A) and XLBR (B). The distinct polypeptide bands (A, lane 4) labeled by the dLBR antibodies in the relative molecular weight range M_r 60,000 to M_r 200,000 represent oligomeric complexes of the dLBR that had been formed at the sperm chromatin during incubation. The XLBR also forms some oligomeric complexes in the presence of sperm chromatin. Arrows (A,B) mark non-aggregated LBR. Molecular masses of reference proteins (in kDa) are marked.

Search for sterol-C14-reductase activity of the dLBR

The hydrophobic C-terminal domain of the vertebrate LBR shares extensive structural similarities with members of the sterol reductase family, including the *S. cerevisiae* sterol C14 reductase, and it has been shown that the hLBR has sterol C14 reductase activity in an *erg24* mutant yeast strain lacking this enzymatic activity (Silve et al., 1998). A BLAST search against Flybase with the ERG24 protein of *S. cerevisiae* (accession number M99419) reveals that, of all predicted *Drosophila* open reading frames, the dLBR exhibits the highest degree of similarity to this sterol C14 reductase (score: 177, 1.5×10^{-19}). Two other *Drosophila* proteins had much lower similarities to the ERG24 protein: a protein phosphate (gene CG3530; score: 81, 0.41) and tetraspanin, a transmembrane protein expressed in axons (gene CG4591; score: 70, 0.94). All other *Drosophila* proteins exhibited no significant similarities to the ERG24 protein (scores: 45, 0.999). These data indicating that no protein comparable in size and secondary structure to the ERG24 protein of *S. cerevisiae* is expressed in *D. melanogaster*.

To clarify whether or not the dLBR can function as a sterol C14 reductase, we transformed an *S. cerevisiae erg24* mutant strain with an expression vector containing the cDNA of the dLBR, the *S. cerevisiae ERG24* gene, a cDNA of the sterol-C14-reductase gene of *Arabidospsis thaliana* (*FACKEL*) (Schrick et al., 2000) or the empty vector (Fig. 7). The immunoblot of total yeast proteins with dLBR antibodies revealed that this *Drosophila* protein is expressed in the transformed yeast strain (Fig. 7A, lane 1). Immunoreactive polypeptides with mobility of dLBR were not detectable in any other strains (Fig. 7A, lanes 2, 3).

Next, we extracted the sterols from these transformed *erg24* strains and analyzed them by combined gas chromatography and mass spectrometry (Fig. 7B-G). Under standard growth conditions, the untransformed *erg24* mutant and the mutant transformed with the empty vector produce ignosterol (ergosta-8,14-dien-3β-ol; Fig. 7E,F) instead of ergosterol (Fig. 7B) as the major end product of the sterol biosynthetic pathway. The *erg24* mutant transformed with *ERG24* (Fig. 7C) or *FACKEL* (Fig. 7D) cDNA reinstated the ability to synthesize ergosterol (Fig. 7B-D). By contrast, the *erg24* mutant expressing dLBR produces only ignosterol as the major end product of the sterol biosynthetic pathway (Fig. 7G; compare with 7E,F). Our data indicate that the *Drosophila* LBR is not able to complement the sterol C14 reductase activity lacking in the *erg24* mutant.

Silencing of dLBR by RNA interference

To gain insight into interactions and functions of the dLBR in vivo, we downregulated its mRNA and the protein in cultured cells (Fig. 8) and embryos (Fig. 9) by RNAi, a method that works very efficiently in *Drosophila* (Clemens et al., 2000). In parallel, we performed RNAi experiments for lamin Dm0, because our data indicate that this lamin binds to dLBR. When an S2 cell culture was transfected with dsRNA of the *dLBR* gene, we noticed by immunofluorescence microscopy with dLBR antibodies a significantly weaker staining of several cells within 24 hours. At 72 hours after transfection, 80-90% of the cells were either not stained or only weakly stained by the antibodies (data not shown; Fig. 8C, Fig. 9B). Identical results were obtained with dsRNA of the lamin *Dm0* gene (data not shown). The number of the weakly stained cells stained by the dLBR antibodies increased within the next days and, 6 days after transfection, most of the cells were indistinguishable from control cells by immunofluorescence microscopy.

To get more quantitative data about the depletion of dLBR (Fig. 8A) and lamin Dm0 (Fig. 8B), we analyzed total cellular proteins of the same number of experimental and control S2 cells by immunoblotting 3 days after transfection. Our results demonstrate that the cell populations transfected with dsRNA of the dLBR contained much less of the dLBR (Fig. 8A, lanes 3,4) whereas the total amount of other proteins like lamin Dm0 (Fig. 8A′) and tubulin (Fig. 8A″) had not been influenced. A directly comparable result was obtained with the lamin *Dm0* dsRNA (Fig. 8B). Lamin Dm0 was specifically depleted (Fig. 8B, lane 2), whereas the total amounts of dLBR (Fig. 8B′) and tubulin (Fig. 8B″) were not affected.

Next, we wanted to know whether the depletion of the dLBR could influence the intracellular distribution of other nuclear envelope proteins. Transfected cells that were barely stained by dLBR antibodies (Fig. 8C) exhibited a localization of lamin Dm0 that was indistinguishable from cells that contained no reduced amounts of the dLBR (Fig. 8C). Our data indicate that the dLBR is not required for the localization and retention of lamin Dm0 in the lamina. In dLBR-depleted S2 cells, the

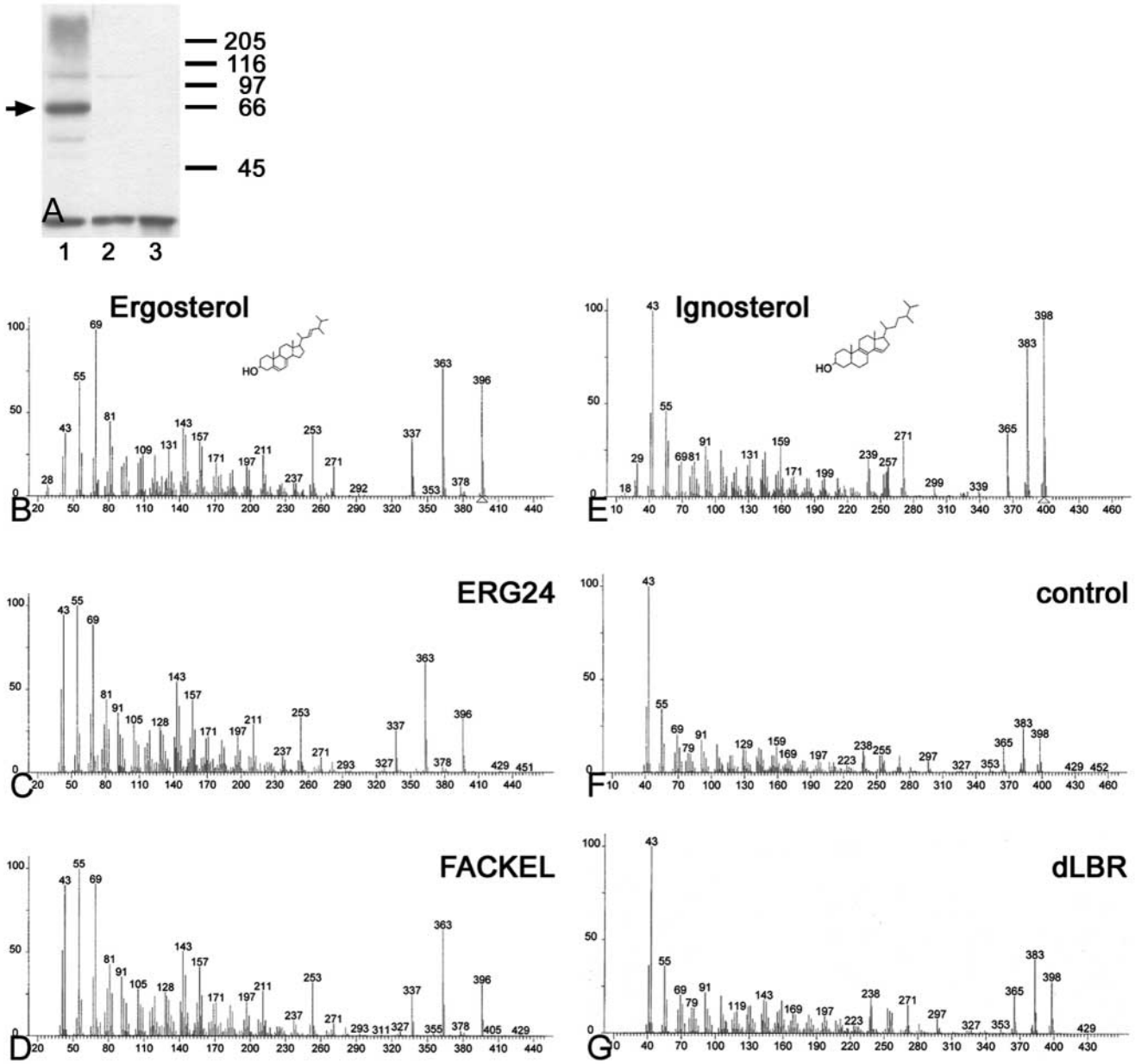


Fig. 7. Expression of the dLBR in the *S. cerevisiae erg24* mutant and analysis of synthesized sterols. (A) *erg24* mutant cells were transformed with plasmids containing the coding region of dLBR (lane 1), the wild-type *ERG24* gene (lane 2) or the plasmid without gene (lane 3). Total proteins of these yeast strains were separated by SDS-PAGE and immunoblotted with dLBR antibodies. The dLBR expressed in yeast forms aggregates in SDS sample buffer; the position of unaggregated dLBR is marked by an arrow. The dLBR antibodies cross-react in addition with a low molecular weight yeast protein present in all three strains. Molecular masses of reference proteins (in kDa) are marked. (B-G) Mass spectrometric analysis of sterols synthesized in *S. cerevisiae erg24* mutant cells that had been transformed with a plasmid containing cDNAs of the following genes: *S. cerevisiae ERG24* (C); *Arabidopsis thaliana FACKEL* (D); a plasmid without gene (F, control); and the *Drosophila* LBR (G, *dLBR*). Sterols were extracted from cells that had been grown for 24 hours in YPAD medium. The mass spectra of ergosterol (B) and ignosterol (E) are shown as standards.

distribution of pore complexes was also not influenced and indistinguishable from control cells. Similar results were obtained with *Drosophila* Kc167 cells (data not shown).

To investigate whether lamin Dm0 is required for the proper localization of the dLBR to the nuclear lamina, we analyzed S2 cells (Fig. 8D,E) and Kc167 cells (Fig. 8F,G) that had been transfected with lamin *Dm0* dsRNA. Silencing of lamin Dm0 in this way led to an altered nuclear morphology of S2 and Kc167 cells. Residual lamin Dm0 was often aggregated in a small dot- or crescent-like structure, on one side of the nucleus (Fig. 8D-G). When lamin Dm0-depleted cells were stained with an antibody (mab414) that specifically reacts with a group of nuclear pore complex proteins (nucleoporins), we noted, in contrast to control cells, an irregular staining of the nuclear periphery. In several cells, areas in the nuclear envelope were stained by the nucleoporin antibodies that apparently contained

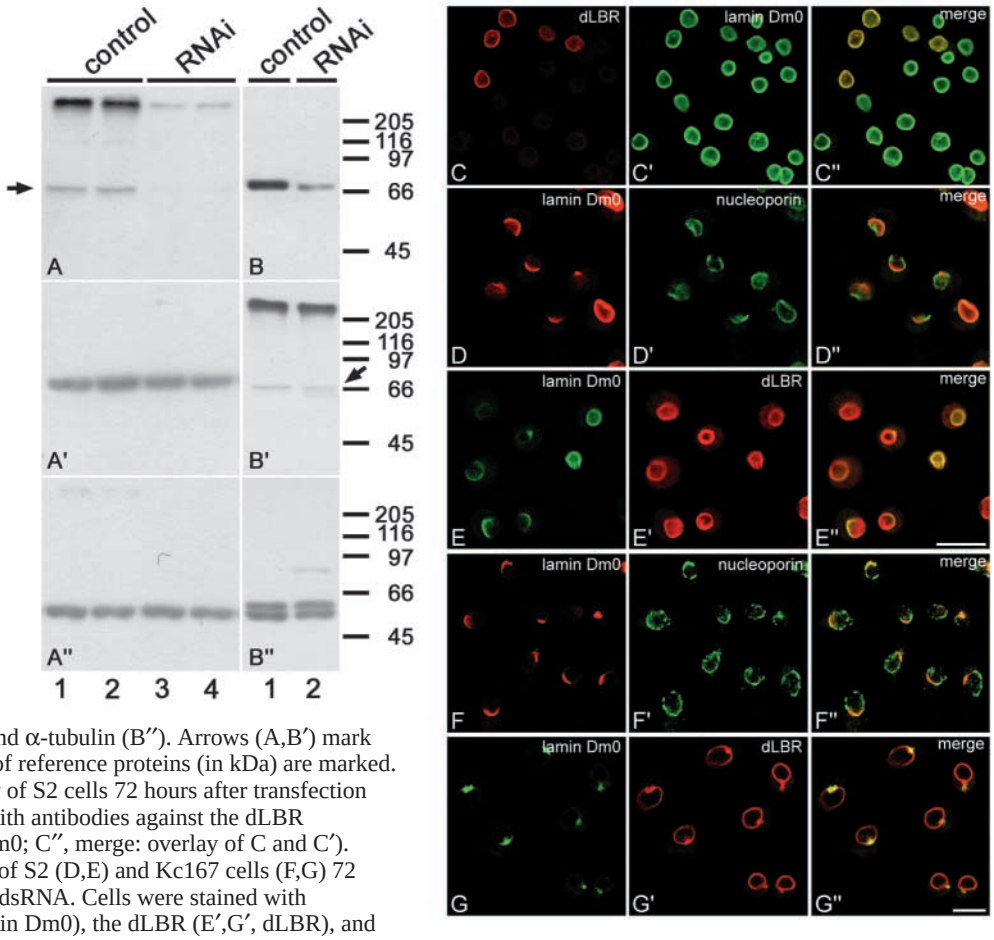


Fig. 8. Downregulation of the *Drosophila* proteins dLBR and lamin Dm0 in cultured *Drosophila* cells by RNAi (A-G). *Drosophila* S2 (A-E) and Kc167 cells (F,G) were transfected with dsRNA specific for the *dLBR* gene (A,C) and the lamin *Dm0* gene (B,D-G), and analyzed by immunoblotting (A,B) and immunofluorescence microscopy (C-G). (A) RNAi of the dLBR. Total proteins of identical numbers of untreated control S2 cells (lanes 1, 2; control) and of S2 cells 3 days after transfection with *dLBR* dsRNA (lanes 3,4; RNAi) were separated by SDS-PAGE and immunoblotted with antibodies against the dLBR (A), lamin Dm0 (A′) and α-tubulin (A″). (B) RNAi of lamin Dm0. Total proteins of identical numbers of untreated control S2 cells (lane 1; control) and of S2 cells 3 days after transfection with dsRNA for the lamin *Dm0* gene (lane 2; RNAi) were separated by SDS-PAGE and immunoblotted with antibodies against lamin Dm0 (B), the dLBR (B′) and α-tubulin (B″). Arrows (A,B′) mark unaggregated dLBR. Molecular masses of reference proteins (in kDa) are marked. (C-C″) Immunofluorescence microscopy of S2 cells 72 hours after transfection with *dLBR* dsRNA. Cells were stained with antibodies against the dLBR (C, dLBR) and lamin Dm0 (C′, lamin Dm0; C″, merge: overlay of C and C′). (D-G) Immunofluorescence microscopy of S2 (D,E) and Kc167 cells (F,G) 72 hours after transfection with lamin *Dm0* dsRNA. Cells were stained with antibodies against lamin Dm0 (D-G, lamin Dm0), the dLBR (E′,G′, dLBR), and antibody mab414 that is specific for nuclear pore complex proteins (D′,F′, nucleoporin). The corresponding overlays are shown (D″-G″, merge). Digital images were taken by CLSM. Scale bars, 20 μm (in E″ for C-E″; in G″ for F-G″).

only very low amounts of lamin Dm0 (Fig. 8D,F). This alteration was most obvious in Kc167 cells (Fig. 8F). The electron microscopic inspection of lamin Dm0-depleted S2 and Kc167 cells revealed phenotypes similar to those described previously for the lamin Dm0 mutant (Lenz-Böhme et al., 1997). We observed aggregates of pore complexes in some areas of the nuclear envelope, whereas other regions of the nuclear membrane were free of pores. In pore-free areas, the outer and inner nuclear membranes were much more distant from each other than in the vicinity of pore complexes and were occasionally ruptured. The nucleoplasm contained irregularly shaped vesicles that appeared to be derived from the inner nuclear membrane (data not shown).

In contrast to the patchy distribution of lamin Dm0 and nuclear pores in lamin-Dm0-depleted cells, the nuclear envelope was homogeneously stained by dLBR antibodies (Fig. 8E′,G′). In addition, we observed that the dLBR localized with dot-like lamin aggregates in the nuclear periphery of Kc167 cells (Fig. 8G,G′). These results indicate that lamin Dm0 supports the localization of dLBR but that it is not essential for the retention of dLBR in the inner nuclear membrane. The depletion of another well-characterized inner nuclear membrane protein by RNAi, *Drosophila* otefin, in S2 and Kc167 cells also did not affect the localization of the dLBR (data not shown).

It has been shown that some cellular proteins including the lamina proteins emerin and lamin A are dispensable in cultured cells (Harborth et al., 2001) but are essential in multicellular organisms (for reviews, see Gruenbaum et al., 2003; Worman and Courvalin, 2002). Because the same could be the case for the dLBR, we depleted this protein by the microinjection of *dLBR* dsRNA into *Drosophila* embryos at 30 minutes old. Squash preparations of whole embryos were made 24 hours and 48 hours after microinjection, and stained with antibodies against the dLBR and lamin Dm0 (Fig. 9). Embryos microinjected with *dLBR* dsRNA were not significantly retarded in their development compared with uninjected control embryos (Table 3). Immunofluorescence microscopy revealed that all embryonic cells of all analyzed embryos at the age of 24 (Fig. 9B) and in most of the 48-hour-old embryos (Table 3) were only very faintly, if at all, stained by dLBR antibodies compared with control embryos of the same age (Fig. 9A). The staining of the nuclei by dLBR antibodies of experimental embryos was only visible when the digital images were recorded with a signal enhancement of more than 760 V (Fig. 9B′; 774 V) but not with a signal enhancement (647 V; Fig. 9B) that has been used for recording of images from control embryos (Fig. 9A,A′, image of the control embryo recorded at 774 V). At 48 hours, in six out of 20 analyzed embryos, a small proportion of cells were stained by dLBR

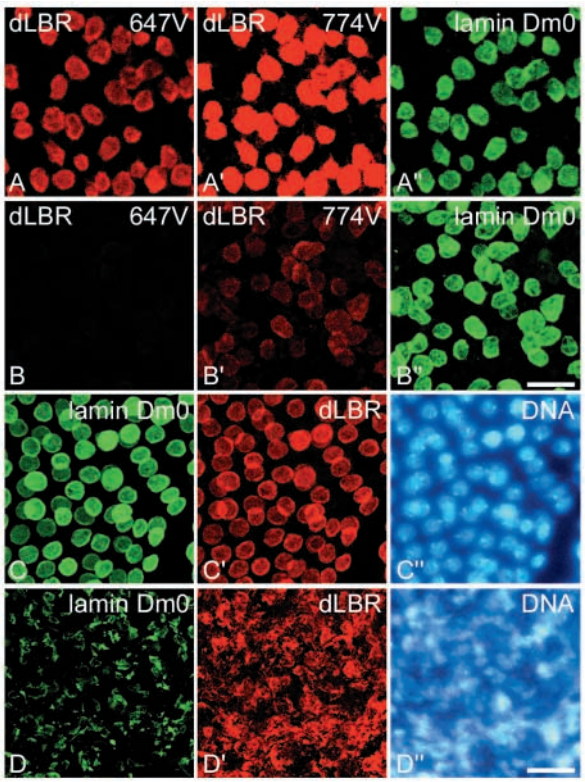


Fig. 9. Depletion of dLBR and lamin Dm0 in *Drosophila* embryos by the microinjection of dsRNA. Embryos at 30 minutes old were microinjected with dsRNA specific for the *dLBR* gene (B-B″, dLBR; A-A″, uninjected control embryo) or lamin Dm0 (D-D″, lamin Dm0; C-C″, uninjected control embryo). Squash preparations of 24-hour-old control and microinjected embryos were stained with antibodies against dLBR (dLBR, A,A′,B,B′,C′,D′) or lamin Dm0 (A″,B″,C,D) and the chromatin was stained by Hoechst 33258 (C″,D″, DNA). To demonstrate the degree of the reduction of the nuclear staining of embryos microinjected with *dLBR* dsRNA, digital images were recorded with a signal enhancement of 647 V (A,B) and 774 V (A′,B′). Weak nuclear staining by dLBR antibodies of embryos microinjected with dLBR dsRNA was first visible at a signal enhancement of 774 V (B′). Most of the nuclei shown in D-D″ were ruptured during the squash preparation owing to the depletion of lamin Dm0 (for quantitative data, see Table 3). Digital images were taken by CLSM (A-B″,C,C′,D,D′) and with a Zeiss Axiophot (C″,D″). Scale bars, 10 μm.

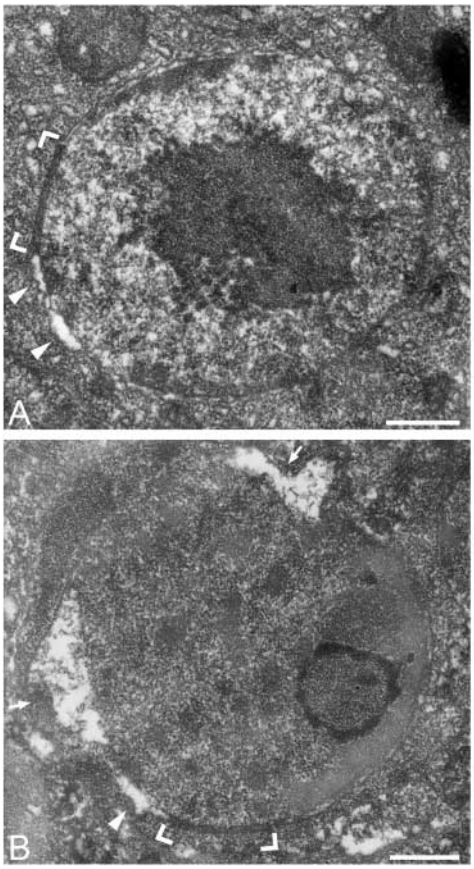


Fig. 10. Electron microscopy of *Drosophila* embryos 48 hours after the microinjection with dsRNA specific for lamin *Dm0*. Electron micrographs of ultrathin sections show two nuclei with altered morphology (A,B) showing clustered pore complexes in the nuclear envelope (brackets) and areas where the outer and inner nuclear membrane has been separated (arrowheads) or where the nuclear envelope is ruptured (A, arrows). Scale bars, 0.5 μm.

antibodies with an intensity close to that of control embryos (Table 3). This result suggests that dLBR synthesis is no longer inhibited in these cells. The lamin Dm0 staining of RNAi-treated embryos and controls was identical and directly comparable with the results obtained for S2 cells, indicating that the depletion of dLBR did not alter the expression and subcellular localization of lamin Dm0 in this multicellular organism.

When we microinjected identical amounts of lamin *Dm0* dsRNA per embryo, their development was arrested at stage 10 (Table 3). Squash preparations revealed that the nuclei of these embryos were very fragile, resulting in the release of the chromatin (Fig. 9D″) compared with control embryos (Fig. 9C″). The immunostaining with lamin Dm0 and dLBR

Table 3. Analysis of *Drosophila* embryos microinjected with dsRNA

	24 hours				48 hours			
	Stage	–	±	+*	Stage	–	±	+*
Controls, not injected	12	–	–	–	First instar	–	–	–
RNAi of *dLBR*, staining with dLBR antibodies	12	8	–	–	17 to first instar	14	6	–
RNAi of lamin *Dm0*, staining with lamin Dm0 antibodies	10	–	–	16	10	–	–	16

Column headings: –, nuclei not stained in these embryos; ±, some but not all nuclei are stained in these embryos; +*, nuclear fragments are stained in these embryos.

The developmental stages reached by 24 hours and 48 hours after microinjection are listed. Embryos were analyzed at the given time point by indirect immunofluorescence microscopy.

antibodies (Fig. 9D,D′) revealed that the nuclear envelope of most embryonic nuclei had been ruptured during the preparation. Many more structures are stained in Fig. 9D′ than in Fig. 9D, indicating that several nuclei contain very little lamin. Similar data were obtained with 48-hour-old embryos (data not shown; Table 3). Electron microscopic inspection of 48-hour-old embryos revealed morphological alterations of the nuclear envelope that had been observed in *Drosophila* cultured cells after transfection with lamin *Dm0* dsRNA. These are aggregation of pore complexes, separation of the outer and inner nuclear membrane, and the local rupture of the nuclear envelope (Fig. 10).

Discussion

We have demonstrated that the predicted open reading frame of the *Drosophila* gene CG17952 encodes a protein that exhibits significant similarities with the vertebrate LBR and so we propose to name this protein *Drosophila* lamin B receptor (dLBR). We show that the common shared properties of the vertebrate LBR and dLBR include their secondary structures (with eight membrane-spanning segments in the C-terminal half of the molecule), the presence of binding domains for sperm chromatin and B-type lamins in the N-terminal domain, and their localization to the inner nuclear membrane. Interestingly, our SDS-PAGE analysis shows that the dLBR also has a physical property in common with the LBRs of *Xenopus* (Gajewski and Krohne, 1999) and chicken (Smith and Blobel, 1993): all three polypeptides exhibit a much higher mobility than expected from their calculated molecular weights (for dLBR, M_r 66,000 instead 83,000 Da).

Our RNAi experiments indicate that the antibodies we have used for the biochemical and immunocytological characterization of the dLBR antibodies are highly specific. Cells or embryos that had been treated with dLBR dsRNA were not or only very weakly stained by dLBR antibodies, and all immunoreactive polypeptides present in control cells were greatly reduced in extracts of RNAi-treated cells. If additional antibodies against other nuclear or cytoplasmic proteins could be contained in our dLBR sera, we would have recognized these activities immediately in RNAi-treated cells. For example, RNAi allowed to distinguish between the specific staining of some antibodies raised against the nuclear pore complex protein Tpr and their cross reaction with unrelated nuclear proteins (Kuznetsov et al., 2002).

Putative functions of the dLBR

The depletion of more than 90% of the dLBR in cultured cells and in embryos by RNAi did not affect the morphology of nuclei and the nuclear envelope, the distribution of other nuclear envelope components, or development during the first 48 hours after fertilization. This result and the observation that, in the *Xenopus* oocyte nuclear envelope, the amount of the LBR is under the level of detection by immunofluorescence microscopy (Gajewski and Krohne, 1999) support the notion that the LBRs of *Drosophila* and vertebrates do not contribute to the mechanical stability of the nuclear envelope. The integrity of the nuclear envelope was also not affected when other inner nuclear membrane proteins, including emerin, LAP2 (Harborth et al., 2001) and otefin (N. Wagner and G. Krohne, unpublished) (Ashery-Padan et al., 1997), were depleted by RNAi. By contrast, a comparable reduction of lamin Dm0 (this paper) (Lenz-Böhme et al., 1997), the lamin of *Caenorhabditis elegans* (Liu et al., 2000; Cohen et al., 2002) or lamin B1 or lamin B2 of mammals (Harborth et al., 2001) caused fragility of the nuclear envelope, cell death and developmental arrest (for review, see Gruenbaum et al., 2003).

It is not known whether the dLBR is an essential protein because no mutant is available. We have verified that a fly stock from the Bloomington Stock Center [stock number 11341; l(2)03605] does contain a single P-element insertion 990 bp 5′ of the start codon of gene CG17952 that causes homozygous lethality during larval and pupa development. However, larvae homozygous for the P-element insertion express normal levels of the dLBR, indicating that the expression of gene CG17952 has not been altered in this fly line (N. Wagner and G. Krohne, unpublished). The mouse LBR mutant ic^J (Shultz et al., 2003) causes developmental abnormalities and a reduced survival in approximately 40-50% of homozygous embryos. Analysis of the homozygous LBR mutants in mice (ic^J mutant) (Shultz et al., 2003) and human (a 17-week-old embryo with HEM/Greenberg skeletal dysplasia) (Waterham et al., 2003) suggest that the vertebrate LBR is not essential for early embryonic development.

The electron microscopic inspection of dLBR-depleted cultured cells of *Drosophila* did not reveal any differences in the nuclear morphology compared with control cells. For the human and the mouse LBR, it has been shown that the reduced expression of the LBR or its absence causes the clumping of chromatin in lymphocytes, intestinal epithelial cells and granule cells of the cerebellum (Schultz et al., 2003). In addition, the mammalian LBR appears to influence the shape of the nucleus in granulocytes. The nuclei of neutrophil granulocytes were only bilobulate or spherical in the reported mouse LBR mutant (Shultz et al., 2003) and in patients with a mutation in the *LBR* gene (Pelger Huet anomaly, HEM/Greenberg skeletal dysplasia) (Hoffmann et al., 2002; Waterham et al., 2003) whereas multisegmented nuclei are characteristic for mammals expressing the wild-type LBR.

dLBR and lipid metabolism

Insects belong to a group of animals that are unable to synthesize sterols de novo (Silberkang et al., 1983). Nevertheless, *Drosophila* expresses dLBR, a protein that exhibits significant similarity to the sterol C14 reductase of the yeast *S. cerevisiae*. Our data indicate that dLBR, in contrast to hLBR (Silve et al., 1998), does not possess sterol C14 reductase activity. In this respect, it is interesting that the C-terminal domain of the dLBR (amino acids 301-741) exhibits only 17% identity to ERG24, the sterol C14 reductase of *S. cerevisiae*, whereas the same region of the hLBR shows 41% identity with the ERG24 protein. Comparative genome analysis of *D. melanogaster* with *Anopheles gambiae* suggests that, during evolution, both insects have lost most of the genes involved in the sterol metabolism, including genes that are required for the ergosterol synthesis (Zdobnov et al., 2002). These genes are present in other organism like *Arabidopsis thaliana*, *S. cerevisiae* and mammals (for review, see Zdobnov et al., 2002). Our data suggest that the sterol C14 reductase activity of the *Drosophila* LBR had been lost during evolution.

Currently, it is not known whether the *Anopheles gambiae* genome codes for a LBR. In the published *Anopheles* genome, we detected a putative protein of 442 amino acids with significant similarity to sterol reductases and the C-terminal segment of the dLBR (peptide ENSANGP00000015268). Other invertebrates that cannot synthesize sterols, like the nematode *C. elegans* (Kurzchalia and Ward, 2003), does not have genes with similarities to sterol reductases, the dLBR and the vertebrate LBRs.

Presently, it is not clear whether the dLBR can mediate additional functions in respect to lipid metabolism that are different from those known for the vertebrate LBR. There is at least one other example illustrating how conserved proteins in *Drosophila* and mammals that are involved in the lipid synthesis have evolved divergent functions. Sterol-regulatory-element-binding proteins (SREBPs) and their interacting partners (for review, see Rawson, 2003) are required for the regulated synthesis of lipids. Via a feedback inhibition, cholesterol regulates the sterol synthesis in mammals through the proteolytic processing of SREBPs, whereas the same pathway in *Drosophila* is regulated by phosphatidylethanolamine and results in the synthesis of membrane lipids that are not sterols (Dobrosotskaya et al., 2002). By analogy, future studies will investigate a possible lipid-related function of the C-terminal domain of dLBR.

We thank M. Paulin-Levasseur, K. Schrick, M. Alsheimer and R. Benavente for critical reading of the manuscript. Yeast plasmids (*ERG24*-pYX212, *FACKEL*-pYX212 and control pYX212) and the *S. cerevisiae erg24* mutant strain were kindly provided by K. Schrick (Keck Graduate Institute, Claremont, CA, USA) and M. Bard (Indiana University-Purdue University Indianapolis, IN, USA), respectively. *Xenopus* egg extracts and sperm chromatin were kindly provided by F. Vollmar and M.-C. Dabauvalle. We thank T. Schmitt for help with the sterol analysis. This work has been supported by a grant to G.K. (DFG KR758/8-4).

References

Ashery-Padan, R., Ulitzur, N., Arbel, A., Goldberg, M., Weiss, A. M., Maus, N., Fisher, P. A. and Gruenbaum, Y. (1997). Localization and posttranslational modifications of otefin, a protein required for vesicle attachment to chromatin, during *Drosophila melanogaster* development. *Mol. Cell. Biol.* **17**, 4114-4123.

Clemens, J. C., Worby, C. A., Simonson-Leff, N., Muda, M., Maehama, T., Hemmings, B. A. and Dixon, J. E. (2000). Use of double-stranded RNA interference in *Drosophila* cell lines to dissect signal transduction pathways. *Proc. Natl. Acad. Sci. USA* **97**, 6499-6503.

Cohen, M., Lee, K. K., Wilson, K. L. and Gruenbaum, Y. (2001). Transcriptional repression, apoptosis, human disease and the functional evolution of the nuclear lamina. *Trends Biochem. Sci.* **26**, 41-47.

Cohen, M., Tzur, Y. B., Neufeld, E., Feinstein, N., Delannoy, M. R., Wilson, K. L. and Gruenbaum, Y. (2002). Transmission electron microscope studies of the nuclear envelope in *Caenorhabditis elegans* embryos. *J. Struct. Biol.* **140**, 232-240.

Collas, P. and Courvalin, J.-C. (2000). Sorting nuclear membrane proteins at mitosis. *Trends Cell Biol.* **10**, 5-8.

Cordes, V., Waizenegger, I. and Krohne, G. (1991). Nuclear pore complex glycoprotein p62 of *Xenopus laevis* and mouse. cDNA cloning and identification of its glycosylated regions. *Eur. J. Cell Biol.* **55**, 31-47.

Dechat, T., Vlcek, S. and Foisner, R. (2000). Lamina-associated polypeptide 2 isoforms and related proteins in cell cycle-dependent nuclear structure dynamics. *J. Struct. Biol.* **129**, 335-345.

Dobrosotskaya, I. Y., Seegmiller, A. C., Brown, M. S., Goldstein, J. L. and Rawson, R. B. (2002). Regulation of SREBP processing and membrane lipid production by phospholipids in *Drosophila. Science* **296**, 879-883.

Dreger, C. K., König, A. R., Spring, H., Lichter, P. and Herrmann, H. (2002). Investigation of nuclear architecture with a domain-presenting expression system. *J. Struct. Biol.* **140**, 100-115.

Duband-Goulet, I. and Courvalin, J.-C. (2000). Inner nuclear membrane protein LBR preferentially interacts with DNA secondary structures and nucleosomal linker. *Biochemistry* **39**, 6483-6488.

Ellenberg, J., Siggia, E. D., Moreira, J. E., Smith, C. L., Presley, J. F., Worman, H. J. and Lippincott-Schwartz, J. (1997). Nuclear membrane dynamics and reassembly in living cells: targeting of an inner nuclear membrane protein in interphase and mitosis. *J. Cell Biol.* **138**, 1193-1206.

Gajewski, A. and Krohne, G. (1999). Subcellular distribution of the *Xenopus* p58/lamin B receptor in oocytes and eggs. *J. Cell Sci.* **112**, 2583-2596.

Gieffers, C. and Krohne, G. (1991). In vitro reconstitution of recombinant lamin A and a lamin A mutant lacking the carboxy-terminal tail. *Eur. J. Cell Biol.* **55**,191-199.

Gietz, D., St Jean, A., Woods, R. A. and Schiestl, R. H. (1992). Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res.* **20**, 1425.

Gruenbaum, Y., Goldman, R. D., Meyuhas, R., Mills, E., Margalit, A., Fridkin, A., Dayani, Y., Prokocimer, M. and Enosh, A. (2003). The nuclear lamina and its functions in the nucleus. *Int. Rev. Cytol.* **226**, 1-62.

Harborth, J., Elbashir, S. M., Bechert, K., Tuschl, T. and Weber, K. (2001). Identification of essential genes in cultured mammalian cells using small interfering RNAs. *J. Cell Sci.* **114**, 4557-4565.

Hoffmann, K., Dreger, C. K., Olins, A. L., Olins, D. E., Shultz, L. D., Lucke, B., Karl, H., Kaps, R., Muller, D., Vaya, A. et al. (2002). Mutations in the gene encoding the lamin B receptor produce an altered nuclear morphology in granulocytes (Pelger-Huet anomaly). *Nat. Genet.* **31**, 410–414.

Holmer, L., Pezhman, A. and Worman, H. J. (1998). The human lamin B receptor/sterol reductase multigene family. *Genomics* **54**, 469-476.

Kawahire, S., Takeuchi, M., Gohshi, T., Sasagawa, S., Shimada, M., Takahashi, M., Abe, T. K., Ueda, T., Kuwano, R., Hikawa, A. et al. (1997). DNA cloning of nuclear localization signal binding protein NBP60, a rat homologue of lamin B receptor, and identification of binding sites of human lamin B receptor for nuclear localization signals and chromatin. *J. Biochem.* **121**, 881-889.

Klapper, M., Exner, K., Kempf, A., Gehrig, C., Stuurman, N., Fisher, P. A. and Krohne, G. (1997). Assembly of A- and B-type lamins studied in vivo with the baculovirus system. *J. Cell. Sci.* **110**, 2519-2532.

Krohne, G., Stuurman, N. and Kempf, A. (1998). Assembly of *Drosophila* lamin Dm0 and C mutant proteins with the baculovirus system. *Eur. J. Cell Biol.* **77**, 276-283.

Kuznetsov, N. V., Sandblad, L., Hase, M., Hunziker, A., Hergt, M. and Cordes, V. C. (2002). The evolutionary conserved single-copy gene for murine Tpr encodes one prevalent isoform in somatic cells and lacks paralogs in higher eukaryotes. *Chromosoma* **111**, 236-255.

Kurzchalia, T. V. and Ward, S. (2003). Why do worms need cholesterol? *Nat. Cell Biol.* **5**, 684-688.

Lang, C. and Krohne, G. (2003). Lamina-associated polypeptide 2β (LAP2 β) is contained in a protein complex together with A- and B-type lamins. *Eur. J. Cell Biol.* **82**, 143-153.

Lang, C., Paulin-Levasseur, M., Gajewski, A., Alsheimer, M., Benavente, R. and Krohne, G. (1999). Molecular characterization and developmentally regulated expression of *Xenopus* lamina-associated polypeptide 2 (XLAP2). *J. Cell Sci.* **112**, 749-759.

Lenz-Böhme, B., Wismar, J., Fuchs, S., Reifegerste, R., Buchner, E., Betz, H. and Schmitt, B. (1997). Insertional mutation of the *Drosophila* nuclear lamin *Dm0* gene results in defective NEs, clustering of nuclear pore complexes, and accumulation of annulate lamellae. *J. Cell Biol.* **137**, 1001-1016.

Liu, J., Ben-Shaharm, T. R., Riemer, D., Treinin, M., Spann, P., Weber, K., Fire, A. and Gruenbaum, A. (2000). Essential role for *Caenorhabditis elegans* lamin gene in nuclear organization, cell cycle progression, and spatial organization of nuclear pore complexes. *Mol. Biol. Cell* **11**, 3937-3947.

Lourim, D. and Krohne, G. (1993). Membrane-associated lamins in *Xenopus* egg extracts: identification of two vesicle populations. *J. Cell Biol.* **123**, 501-512.

Meier, J. and Georgatos, S. (1994). Type B lamins remain associated with the integral nuclear envelope protein p58 during mitosis: implications for nuclear reassembly. *EMBO J.* **13**, 1888-1898.

Polioudaki, H., Kourmouli, N., Drosou, V., Bakou, A., Theodoropoulos, P. A., Singh, P. B., Giannakouros, T. and Georgatos, S. D. (2001). Histones

H3/H4 form a tight complex with the inner nuclear membrane protein LBR and heterochromatin protein 1. *EMBO Rep.* **21**, 920-925.

Pyrpasopoulou, A., Meier, J., Maison, C., Simos, G. and Georgatos, S. D. (1996). The lamin B receptor (LBR) provides essential chromatin docking sites at the nuclear envelope. *EMBO J.* **24**, 7108-7119.

Rawson, R. B. (2003). The SREBP pathway – insights from Insigs and insects. *Nat. Rev. Mol. Cell Biol*. **4**, 631-640.

Schmidt, M., Tschödrich-Rotter, M., Peters, R. and Krohne, G. (1994). Properties of fluorescently labeled *Xenopus* lamin A in vivo. *Eur. J. Cell Biol*. **65**, 70-81.

Schoft, V. K., Beauvais, A. J., Lang, C., Gajewski, A., Prüfert, K., Winkler, C., Akimenko, M.-A., Paulin-Levasseur, M. and Krohne, G. (2003). The lamina-associated polypeptide 2 (LAP2) isoforms β, γ and ω of zebrafish: developmental expression and behavior during the cell cycle. *J. Cell Sci*. **116**, 2505-2517.

Schrick, K., Mayer, U., Horrichs, A., Kuhnt, C., Bellini, C., Dangl, J., Schmidt, J. and Jürgens, G. (2000). FACKEL is a sterol C-14 reductase required for organized cell division and expansion in *Arabidopsis* embryogenesis. *Genes Dev.* **14**, 1471-1484.

Shultz, L. D., Lyons, B. L., Burzenski, L. M., Gott, B., Samuels, R., Schweitzer, P. A., Dreger, C., Herrmann, H., Kalscheuer, V., Olins, A. L. et al. (2003). Mutations at the mouse *ichtyosis* locus are within the lamin B receptor gene: a single gene model for human Pelger-Hu_t anomaly. *Hum. Mol. Genet.* **12**, 61-69.

Silberkang, M., Havel, C. M., Friend, D. S., McCarthy, B. J. and Watson, J. A. (1983). Isoprene synthesis in isolated embryonic *Drosophila* cells. *J. Biol. Chem.* **10**, 8503-8511.

Silve, S., Dupuy, P. H., Ferrara, P. and Loison, G. (1998). Human lamin B receptor exhibits sterol C14-reductase activity in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* **1392**, 233-244.

Simos, G. and Georgatos, S. D. (1992). The inner nuclear membrane protein p58 associates in vivo with a p58 kinase and the nuclear lamins. *EMBO J.* **11**, 4027-4036.

Smith, S. and Blobel, G. (1993). The first membrane spanning region of the lamin B receptor is sufficient for sorting to the inner nuclear membrane. *J. Cell Biol.* **120**, 631-637.

Soullam, B. and Worman, H. J. (1993). The amino-terminal domain of the lamin B receptor is a nuclear envelope targeting signal. *J. Cell Biol.* **120**, 1093-1100.

Starr, D. A. and Han, M. (2003). ANChors away: an actin based mechanism of nuclear positioning. *J. Cell. Sci.* **116**, 211-216.

Stuurman, N., Sasse, B. and Fisher, P. A. (1996). Intermediate filament protein polymerization: molecular analysis of *Drosophila* nuclear lamin head-to-tail binding. *J. Struct. Biol.* **117**, 1-15.

Takano, M., Takeuchi, M., Ito, H., Furukawa, K., Sugimoto, K., Omata, S. and Horigome, T. (2002). The binding of lamin B receptor to chromatin is regulated by phosphorylation in the RS region. *Eur. J. Biochem.* **269**, 943-953.

Waterham, H. R., Koster, J., Mooyer, P., van Noort, G., Kelley, R. I., Wilcox, W. R., Wanders, R. J. A., Hennekam, R. C. M. and Oosterwijk, J. C. (2003). Autosomal recessive HEM/Greenberg skeletal dysplasia is caused by 3β-hydroxysterol Δ^{14}-reductase deficiency due to mutations in the lamin B receptor gene. *Am. J. Hum. Genet.* **72**, 1013-1017.

Worman, H. J. and Courvalin, J.-C. (2002). The nuclear lamina and inherited disease. *Trends Cell Biol.* **12**, 591-598.

Ye, Q. and Worman, H. J. (1994). Primary structure analysis and lamin B and DNA binding of human LBR, an integral membrane protein of the nuclear envelope inner membrane. *J. Biol. Chem.* **269**, 11306-11311.

Ye, Q. and Worman, H. J. (1996). Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to *Drosophila* HP1. *J. Biol. Chem.* **271**, 14653-14656.

Ye, Q., Callebaut, I., Pezhman, A., Courvalin, J.-C. and Worman, H. J. (1997). Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR. *J. Biol. Chem.* **272**, 14983-14989.

Ye, Q., Barton, R. M. and Worman, H. J. (1998). Nuclear lamin binding proteins. In *Subcellular Biochemistry: Intermediate Filaments* (ed. H. Hermann and J. Robin Harris), pp. 587-610. London, UK: Plenum Publishing.

Zdobnov, E. M., von Mering, C., Letunic, I., Torrents, D., Suyama, M., Copley, R. R., Christophides, G. K., Thomasova, D., Holt, R. A., Subramanian, G. M. et al. (2002). Comparative genome and proteome analysis of *Anopheles gambiae* and *Drosophila melanogaster*. *Science* **298**, 149-159.