Bin3 Deletion Causes Cataracts and Increased Susceptibility to Lymphoma during Aging

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Abstract

Bin3 encodes an evolutionarily conserved and ubiquitously expressed member of the BAR superfamily of curved membrane and GTPase-binding proteins, which includes the BAR, PCH/F-BAR, and I-BAR adapter proteins implicated in signal transduction and vesicular trafficking. In humans, Bin3 maps to chromosome 8p21.3, a region widely implicated in cancer suppression that is often deleted in non–Hodgkin’s lymphoma and various epithelial tumors. Yeast studies have suggested roles for this gene in filamentous actin (F-actin) organization and cell division but its physiologic functions in mammals have not been investigated. Here we report that homozygous inactivation of Bin3 in the mouse causes cataracts and an increased susceptibility to lymphomas during aging. The cataract phenotype was marked by multiple morphologic defects in lens fibers, including the development of vacuoles in cortical fibers and a near total loss of F-actin in lens fiber cells but not epithelial cells. Through 1 year of age, no other phenotypes were apparent; however, by 18 months of age, Bin3<sup>−/−</sup> mice exhibited a significantly increased incidence of lymphoma. Bin3 loss did not affect normal cell proliferation, F-actin organization, or susceptibility to oncogenic transformation. In contrast, it increased the proliferation and invasive motility of cells transformed by SV40 large T antigen plus activated ras. Our findings establish functions for Bin3 in lens development and cancer suppression during aging. Further, they define Bin3 as a candidate for an unidentified tumor suppressor that exists at the human chromosome 8p21.3 locus.

Introduction

BAR adapter proteins, named for a shared sequence motif initially defined in the Bin1, amphiphysin, and yeast RVS proteins (1), function in diverse cellular processes, including membrane dynamics, actin organization, polarity, stress response, antiproliferation, immunity, and tumor suppression (2). The signature domain of this class of proteins, the BAR domain, interacts with and facilitates tubulation of curved membranes and also binds to small GTPases and other cell regulatory proteins in the cytosol and nucleus (3). Recently, crystallographic studies have revealed a BAR superfamily that includes not only BAR adapters but also members of the PCH (F-BAR) and I-BAR adapter families (4). Within the original BAR family subgroup, Bin3 is, along with Bin1/amphiphysin II, one of only two members that are both ubiquitously expressed in mammalian cells and conserved throughout evolution to yeast (5). Studies of the budding and fission yeast homologues, termed Rvs161 and hob3+, have highlighted essential functions in filamentous actin (F-actin) organization and cell division (5–8). Extending these observations, a recent study revealed that in fission yeast Hob3p can recruit the Rho family small GTPase Cdc42 to support its role in polarized cell division (9). However, the physiologic functions of Bin3 in mammals have yet to be investigated in any depth.

One stimulus to investigation of the Bin3 gene is its interesting location at human chromosome 8p21.3 within a region that has been implicated widely in cancer suppression (10). Indeed, losses of chromosome 8p represent one of the most common events in epithelial tumors and B lymphomas and such events have been associated strongly with progression in advanced metastatic disease. In particular, recent fine-mapping studies have highlighted a ~1 Mb region at 8p21.3, including Bin3, as the site of a tumor suppressor gene(s) involved in the development of non–Hodgkin’s lymphoma, head and neck cancer, and prostate adenocarcinoma (11–13). However, among the genes within the region implicated, a clear suppressor has yet to be identified. In support of the notion that Bin3 may be germane, another prototypical BAR family member, Bin1/amphiphysin II, has been shown to function in cancer suppression (1, 14–21). To evaluate the physiologic functions of Bin3 in mammals, we studied the consequences of its genetic deletion in the mouse.

Materials and Methods

Generation and genotyping of Bin3 nullizygous mice. The proximal promoter and exon 1 of the murine Bin3 gene was replaced with a PGK-neo cassette using standard methods for homologous recombination in the mouse. Briefly, a genomic targeting plasmid with the structure noted in Fig. 1A was introduced by electroporation into AB2.1 murine embryonic stem cells. Clones with the desired homologous recombination event were microinjected into C57BL/6 blastocysts and resulting male chimeric animals were bred with C57BL/6 females to obtain offspring with germ-line transmission of the knockout (KO) allele (as identified by Southern blot.
Mice were interbred and maintained on a mixed C57BL/6J-129/SvJ genetic background. PCR was used to genotype mice as follows: Mouse tissue samples were dissociated 1 h at 95°C in lysis buffer (25 mmol/L NaOH plus 0.2 mmol/L EDTA) and then neutralized with equal volume of 40 mmol/L Tris-HCl. DNA-containing supernatant was used for PCR in a volume of 20 μL in a PTC-200 Peltier Thermal Cycler (MJ Research). The primers used were 5'-GTTAGGCCTCAGCTCTCCCTGAAGATTC-3’ or 5'-GCTTGGCTGGATTAACCTCTCTTCAG-3’ and 5'-CTGGGCCTTGACTCCTCATCTATCA-3’ with expected sizes of 423 or 264 bp for wild-type (WT) or nullizygous alleles, respectively. Following a 3-min denaturation at 96°C, 35 cycles of PCR were performed at 96°C for 30 s, 61°C for 30 s, and 72°C for 1.5 min with the addition of a 5-min elongation step at 72°C. All experiments using mice were approved by the Lankenau Institute for Medical Research and University of Delaware Animal Care and Use Committees and they conformed to the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research.

**Cell culture.** Mouse embryonic fibroblasts (MEF) were generated and cultured as described previously (20, 22). COS cells were cultured and transfected in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics. To compare cell proliferation, cells were seeded in triplicate into 100-mm dishes that were uncoated or coated with the nonadherent agent polyHEMA (23), and at the indicated times afterward, cells were counted after trypsinization to determine cell outgrowth. For cell motility as measured by Transwell migration assay, cells were monitored as described (24) in a modified Boyden chamber (8-A μm pore size, two-well Costar Transwell, Corning Life Sciences) according to the manufacturer’s instructions. Briefly, 10⁵ serum-deprived cells were seeded in triplicate in the top wells in medium containing 0.1% FBS, and 48 h later, cells that had migrated into the bottom well containing medium supplemented with 10% FBS were trypsinized and counted.

**Antibody preparation and Western blot analysis.** Recombinant full-length human Bin3 was expressed in bacteria with a COOH-terminal glutathione S-transferase (GST) fusion tag and used to create an anti-Bin3 monoclonal antibody using methods that have been described (25). The resulting hybridoma (3A4) is an IgG2b with a κ light chain and reacts with the highly similar (94% sequence identity) mouse Bin3 on Western blots. MEFs were isolated from WT and Bin3-null mice by standard methods, and cell extracts for Western blot analysis were prepared by harvesting cells, which were washed thrice in PBS, before lysis in 1× radioimmunoprecipitation assay buffer (1× PBS containing 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, and 10 μg/mL phenylmethylsulfonyl fluoride), including 10 μL/mL Protease Inhibitor Set III and Phosphatase Inhibitor Set II (Calbiochem). Protein was quantitated by Bradford assay and 50 μg protein per sample was analyzed by SDS-PAGE. Gels were processed by standard Western blotting methods using a horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibody (1:2,000 dilution; Cell Signaling). For actin, a primary anti-actin goat polyclonal antibody was used (1:500 dilution; Santa Cruz Biotechnology) and HRP-conjugated rabbit anti-goat secondary antibody (1:5,000 dilution; Southern Biotechnology Associates). Antibody detection was carried out using a commercial chemiluminescence kit (Pierce).

**Gross and histologic tissue analysis.** Slit lamp photographs were taken of anesthetized mice by standard methods. Dark field microscopic analysis of the lens phenotype was performed by dissecting lenses from eyes and placing them in Medium 199 with Earle’s salts and l-glutamine (Mediatech), which is isotonic to the lens (26). Lenses were photographed with a dissecting microscope (Stemi SV11 Apo, Carl Zeiss) fitted with a digital camera. The resulting photographs were processed by removing the color information and reducing the brightness in Adobe Photoshop. For histologic analysis, whole eyes were fixed in 4% neutral buffered formalin for
18 h, transferred to 70% ethanol, and stored until paraffin embedding. Sections (6 μm) were prepared, stained with H&E, and photographed on an upright microscope by standard methods.

Phalloidin staining. Lenses were dissected from the eye, fixed for 2 h in 4% neutral buffered formalin, washed thrice for 15 min each in PBS with 0.1% Triton X-100, and stained in PBS with 0.25% Triton X-100, a 1:2,000 dilution of DRAQ5 (Biostatus Ltd.), and 1:200 Alexa Fluor 488–labeled phalloidin (Molecular Probes) overnight at 4°C. Lenses were subjected to three 15-min room temperature washes in 0.1% Triton X-100 in PBS before storage in PBS at 4°C. Whole stained lenses were placed epithelial side down in an uncoated 35-mm #1 glass-bottom culture dish (MatTek Corp.) filled with PBS and imaged in the XY plane with a LSM 510 VIS confocal microscope fitted with a 20× objective lens, a 30-mW argon krypton laser, and a 5-mW helium-neon laser (Carl Zeiss).

Bin3 immunofluorescence microscopy. Bin3 localization was analyzed in COS cells transiently transfected with a hemagglutinin (HA)-tagged Bin3 expression construct. Staining methods were essentially as described above and detailed elsewhere (27, 28). MitoTracker (Molecular Probes) and the DNA dye 4′,6-diamidino-2-phenylindole (DAPI) were used as counterstains to identify mitochondria and nuclei, respectively, in COS cells.

Carcinogenesis. For irradiation, 6- to 8-wk-old mice were exposed to a single sublethal dose of 4 or 7 Gy γ-rays from a 137Cs γ-iradiator and then monitored to an end point of 12 mo when all animals were euthanized. For chemical carcinogenesis, 6- to 8-wk-old mice were subjected to classic protocols of lung, skin, and breast carcinogenesis. For lung carcinogenesis, mice were given as described (29) a single ip dose of diethylnitrosamine (DEN) at 20 or 50 mg/kg body weight. Lung tumors induced in this manner occur with a mean latency of 24 wk in A/J mice (29). Mice treated with DEN were monitored up to an end point of 12 mo, after which all animals were euthanized and examined at necropsy for evidence of tumor formation. For skin carcinogenesis, mice were shaved and the dorsal epidermis was treated essentially as described (30, 31) with a single dose of 400 nmol 7,12-dimethylbenz(a)anthracene (DMBA) followed by a twice-weekly application of 17 nmol phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). Mice treated with DMBA + TPA were monitored to a 20-wk end point for skin tumors. For mammary carcinogenesis, mice implanted with a subdermal medroxyprogesterone pellet were treated with a single ip dose of DMBA and tumor formation was monitored as described (20). All suspected lesions at necropsy were weighed and processed by standard histologic analysis.

Results

Bin3 is nonessential for embryonic development or fertility. The murine Bin3 gene encompasses ∼38 kb and 8 exons on chromosome 14D1-2, encoding a polypeptide composed of a single BAR domain. We used standard methods to replace the proximal promoter and exon 1 of this gene with a PGK-neo cassette (Fig. 1A) by homologous recombination in embryonic stem cells, generating chimeric animals by blastocyst microinjection. Germ-line transmission of the targeted allele was observed in heterozygous animals by Southern blot analysis of genomic DNA from two separate founder mice generated by the same embryonic stem cell population (Fig. 1B), one of which was further characterized. The loss of Bin3 protein in null mice was confirmed by Western blot analysis of primary MEFs isolated from heterozygous or homozygous null embryos using a monoclonal antibody raised against a recombinant GST-Bin3 fusion protein. This antibody detected a ∼31 kDa protein in MEFs+/− but not MEFs−/− that was consistent with the size of the Bin3 polypeptide predicted from its primary structure (Fig. 1C). Somewhat unexpectedly, given the robust defect in cytokinesis produced in fission yeast cells by mutation of the Bin3 homologue hob3+ (5, 9), we found that viable homozygous null mice were readily obtained at Mendelian ratios without any apparent defects in survival or fertility (data not shown). This finding immediately argued that in mammals Bin3 has a nonessential role in cell division, highlighting some functional differences with fission yeast hob3+ despite the homology of these genes (5).

Bin3-null mice develop cataracts soon after birth, which are characterized by severe defects in cytoskeletal F-actin organization. Within a few weeks to a few months of birth, Bin3−/− mice developed obvious gross lenticular opacities in one or both eyes, whereas mice heterozygous or WT for the deletion did not develop cataracts (Fig. 2A–H). This phenotype was highly penetrant, appearing in all nullizygous animals examined by 6 months of age. Histopathologic examination of lenses from 1-week-old Bin3−/− mice (Fig. 2G) did not reveal any obvious defects; however,
by 8 weeks of age, the lens cortex of nullizygous animals had developed numerous large vacuoles (Fig. 2H), although the lens epithelium was apparently unaffected. There was no change in the size of the eye in Bin3−/− mice and the retina, cornea, and other tissues of the eye did not show obvious pathologic features. In contrast, no lens defects were observed in the lens sections obtained from Bin3 heterozygous or WT littermates.

Genetic studies of yeast homologues of Bin3 suggest that this gene functions in F-actin organization (5, 32). Therefore, we analyzed the F-actin organization of lenses from 8-week-old heterozygous and nullizygous mice. In lens fiber cells, F-actin is normally found directly underlying the lateral cell membranes (Fig. 3A). In Bin3−/− lenses, we observed a striking loss of F-actin from the lens fiber cells and the little actin structure that remained was highly disorganized (Fig. 3B). In contrast, F-actin organization in the lens epithelial cells of nullizygous mice seemed to be relatively unaffected. Furthermore, we did not observe any alteration in the actin cytoskeleton of Bin3−/− MEFs (data not shown). Taken together, these observations indicated that Bin3 ablation caused a specific disruption of F-actin structure in lens fiber cells.

Although efforts to visualize endogenous Bin3 by indirect immunofluorescence staining methods were unsuccessful in embryonic or adult murine eye, as well as in other tissue types and MEFs (data not shown), in COS kidney cells where a HA-tagged human Bin3 was expressed transiently, we observed a cytosolic vesicular localization expected for BAR adapter proteins (Fig. 4). Similar results were obtained on expression of WT Bin3 where protein was visualized with Bin3 monoclonal antibody (data not shown). We concluded that Bin3 supported actin organization and likely functioned at cytosolic vesicular membranes in cells. Based on a recent report that fission yeast Hob3p can recruit Cdc42 to support its role in cell division, we compared Cdc42 expression and localization in lenses and embryonic fibroblasts derived from Bin3−/− animals. However, we did not detect any differences in Cdc42 expression or localization in either cell type (data not shown). These findings were not unexpected based on the profound contrast between the phenotypes produced by deletions of Bin3 or Cdc42, which in the latter case causes early embryonic lethality in the mouse associated with gross defects in structural organization and primary ectoderm (33). This strong difference in phenotype clearly pointed to greater degeneracy in the function of Bin3 in mammals compared with fission yeast.

Bin3 suppresses lymphoma during aging and restricts the efficiency of lung carcinogenesis. Other than juvenile cataracts, no other apparent phenotypes were noted in Bin3−/− mice through 1 year of age. In contrast, beyond 1 year of age Bin3−/− mice displayed a significantly elevated incidence of lymphoma, with 36% of Bin3−/− mice exhibiting lymphomas by 18 months of age compared with none of the age-matched heterozygous or WT control animals (Table 1; Fig. 5A). Lymphomas were observed to arise in the lung, liver, intestine, spleen, and lymph nodes. Among
these types of tumors, two were identified specifically as mucosa-associated lymphoid tissue lymphoma and two as follicular lymphoma (data not shown). Bin3+/−/− mice also exhibited modest increases in congestion and inflammation in the lung or liver and also in myelodysplastic syndrome and congestion with extramedullary hemopoiesis in spleen (~15% and ~12% of nullizygous animals, respectively; Table 1; Fig. 5B). Taken together, these observations suggested that Bin3 acted to suppress lymphoma formation during aging.

We reasoned that a negative modifier effect of Bin3 on cancer might also manifest in animals exposed to a carcinogen. To examine this idea, we compared the responses of Bin3+/− mice to γ-irradiation, exposure to a chemical carcinogen, or activation of the c-myc oncogene in a transgenic cross. In these experiments, heterozygous animals were used as controls based on evidence of haplosufficiency of Bin3 for lymphoma suppression and the desire to control for the neomycin cassette present at the homozygous KO locus. These experiments suggested a specific trend for cooperation of Bin3 loss with a facilitation of lung tumor formation. For irradiation, mice of 6 to 8 weeks of age were subjected to a single whole-body dose of 4 to 7 Gy γ-radiation from a 137Cs source and monitored to a 12-month end point. Although the penetrance of c-myc-mediated tumor formation during aging was limited, the null group exhibited greater sensitivity to the formation of lung adenocarcinoma (Supplementary Table S1; Supplementary Fig. S1C). Although liver tumors did not arise in these animals, 30% of the Bin3+/− mice displayed hepatic congestion and 20% displayed hepatocyte atypia (Supplementary Table S2). Neither of these premalignant lesions occurred in the control group. Finally, we also compared the effects of Bin3 loss in two models of mammary tumorigenesis involving initiation by chemical carcinogen DMBA or overexpression of the c-myc oncogene. Briefly, mice were subjected to a standard protocol of carcinogen treatment or interbred with MMTV-c-myc mice (35) before nullizygous and heterozygous virgin or parous females were compared for tumor incidence, kinetics, and histology. In both models, Bin3 loss did not affect mammary tumorigenesis (data not shown), implying that Bin3 suppressed tumor formation only in specific tissues, only in cooperation with certain oncogenic lesions, or both. In summary, we concluded that Bin3 restricted the efficiency of lung carcinogenesis and suppressed the development of spontaneous lymphomas during aging.

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Bin3+/+ (n = 10)</th>
<th>Bin3+/− (n = 23)</th>
<th>Bin3−/− (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td>0/10 (0%)</td>
<td>0/23 (0%)</td>
<td>13/33 (39%)</td>
</tr>
<tr>
<td>Lung</td>
<td>0/10 (0%)</td>
<td>0/23 (0%)</td>
<td>3/33 (9%)</td>
</tr>
<tr>
<td>Congestion, chronic inflammation</td>
<td>0/10 (0%)</td>
<td>1/23 (4%)</td>
<td>2/33 (6%)</td>
</tr>
<tr>
<td>Liver</td>
<td>0/10 (0%)</td>
<td>1/23 (4%)</td>
<td>2/33 (6%)</td>
</tr>
<tr>
<td>Congestion, chronic inflammation</td>
<td>0/10 (0%)</td>
<td>1/23 (4%)</td>
<td>2/33 (6%)</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>0/10 (0%)</td>
<td>1/23 (4%)</td>
<td>2/33 (6%)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0/10 (0%)</td>
<td>1/23 (4%)</td>
<td>2/33 (6%)</td>
</tr>
<tr>
<td>Congestion, extramedullary hemopoiesis</td>
<td>1/10 (10%)</td>
<td>1/23 (4%)</td>
<td>2/33 (6%)</td>
</tr>
</tbody>
</table>

NOTE: Mice were euthanized at 18 mo of age and examined at necropsy for pathologic evidence of tumor formation. All suspected lesions were confirmed by histologic analysis. *P = 0.0196 (+/+ versus −/−) or 0.0003 (+/+ versus −/+−) using a two-tailed Fisher’s exact test.

Figure 5. Lymphoma and splenic histopathology in aging Bin3+/− mice. Representative lesions scored in nullizygous animals were processed for sectioning and staining with H&E using standard histologic methods. A, lymphoma in liver in an 18-mo-old animal. B, splenic congestion with extramedullary hemopoiesis in an 18-mo-old animal.
Specifically, Bin3 acts as a suppressor gene that restricts the proliferation and migration of transformed primary cells. A, morphology. Cells were photographed in tissue culture at ×200 magnification. B, transgene expression. Expression of SV40 large T antigen and mutant H-Ras in transformed cell populations was confirmed by Western blot analysis using antibodies to the oncoproteins. C, cell proliferation. Cells (10⁶) were seeded into 100-mm dishes and cell number was counted using a hemocytometer after trypansinization at the times indicated. Proliferation was compared on plastic dishes and dishes coated with polyHEMA, a nonadherent substrate. D, invasive motility. Cells (10⁵) were plated in duplicate into modified Boyden chamber plates (8-μm pore size) and the relative number of cells that penetrated to the bottom of the well 48 h later was determined. The mean of the data is shown.

Discussion

The results of this study define essential functions for Bin3 in postnatal lens development and lymphoma suppression during aging. Advanced age is the major risk factor for many diseases, yet most preclinical models use young animals that do not fully recapitulate the participation of inflammation, immune senescence, or other factors that are affected significantly by aging. Insights into the cause and treatment of age-associated diseases might benefit from studies of pathways that modify disease susceptibility during aging. However, few such pathways have been defined.

Cataracts are the leading cause of blindness in the world. Although most cataracts are diagnosed in the elderly, congenital cataracts also occur in infants and children at a rate of ~3 in 10,000 births, accounting for ~10% of vision loss in children worldwide. Congenital cataracts have been found as components of multisystem syndromes, in association with other defects in ocular development, or as isolated anomalies. Many of the genes responsible for human and mouse autosomal dominant cataracts have been discovered in recent years (37) due to the completion of a saturation mutagenesis screen of the mouse genome for loci responsible for dominant cataract (38). However, the etiology of the vast majority of human congenital cataracts remains unknown (39) and may well represent autosomal recessive traits. Mouse models have been used to define genes involved in congenital eye abnormalities, and in recent years, they have advanced understanding of lens morphogenesis, physiology, and the pathogenesis and pathophysiology of cataract (39, 40). One notable aspect of the human chromosomal localization of Bin3 at 8p21.3 is its synteny with the midsection of murine chromosome 14, where a highly penetrant cataract locus, termed rupture of lens cataract (Rlc), has been mapped. Rlc causes lens opacity starting at 1 to 2 months of age with vacuole formation and lens nucleus rupture in advanced cases (41–44). Like Bin3 loss, the effects of Rlc seem to be confined to the lens without effects on development, viability, or fertility. Given their relative proximity and similarities in cataractogenesis, the relationship between Rlc and Bin3 may deserve further attention.

In cancer, chromosome 8p is among the most commonly altered regions of the genome, with a large body of literature implying the presence of at least three tumor suppressor genes. Although the identity of these genes has yet to be identified conclusively, inactivation of one or more is strongly implicated in the genesis of a variety of epithelial and hematologic malignancies. In particular, there is extensive evidence pointing to a suppressor locus 8p21.3 that is germane to the development of non–Hodgkin’s lymphoma (11), prostate cancer (12, 45–48), head, neck, oral, and laryngeal cancers (13, 49), and lung cancers (50). Indeed, recent fine-mapping...
studies of the 8p21.3 region have highlighted a ~1 Mb region containing ~10 genes, including the Bin3 gene, as a focal point of relevance to non-Hodgkin’s lymphoma and prostate adenocarcinoma (11–13). The appearance of lymphomas in Bin3−/− mice is clearly consistent with the possible relevance of Bin3 as a tumor suppressor in this setting. Although we did not see prostate tumors spontaneously arise in Bin3−/− mice, this connection needs to be explored more directly because, based on our observations, one would not expect Bin3 ablation to be sufficient on its own to drive epithelial tumorigenesis in the absence of a cooperating oncogenic stimulus. Although the 8p21-22 region in humans also has been implicated in breast cancer suppression, our results argued against a role for Bin3 in this setting, suggesting either it is irrelevant or that conditions required for relevance were not operative in the models examined. Nevertheless, our findings as a whole clearly support further evaluation of Bin3 as a candidate for the tumor suppressor gene inferred to reside at human chromosome 8p21.3.

Our findings provide a biological foundation for further investigation into how Bin3 functions. During lens fiber cell differentiation, these cells develop elaborated lateral membranes that form high surface area interdigitations with their neighbors to allow for extensive cell communication in this avascular tissue (51). Because these interdigitations also are tightly associated with the actin cytoskeleton (52), this association supports a Bin3 connection in actin cytoskeletal dynamics and membrane structure. However, if this is the case, it remains unclear why the lens phenotype should develop so late, only after eye lid opening. As noted above, yeast homologues of Bin3 have been implicated in F-actin organization, vesicle trafficking, and cell polarity (5–8, 36). Lens fiber cells normally have most of their F-actin distributed directly under cell membrane in lens fiber (53), whereas Bin3−/− lens fiber cells lose much of their F-actin cytoskeleton and what is remaining does not seem to localize along the lateral cell membranes. This phenotype suggests that Bin3 may be essential to maintain the actin cytoskeleton of lens fibers. In support of this notion, Bin3 is able to complement defects in F-actin localization in fission yeast caused by mutation of its homologue hob3+. Alternatively, Bin3 may be important for actin dynamics in the lens by supporting short-term disassembly of actin stress fibers in lens epithelial cells, which seems to be sufficient to induce differentiation in lens fiber cells (54).

A small set of interactions for yeast homologues of Bin3 has been described, but the relevance of two of the best characterized of these interactions remains uncertain in mammals as yet. Studies of the budding yeast homologue Rvs161p indicate a critical reliance on its physical interaction with Rvs167p, the Bin1/amphiphysin orthologue in yeast, yet we have been unable, to date, to obtain any evidence of similar physical interactions between either the fission yeast orthologues Hob1p and Hob3p or the mammalian orthologues Bin1 and Bin3.8 A second important interaction has been reported between Hob3p and Cdc42p (9), a Rho family small GTPase that contributes to polarized cell division and cytokinesis in fission yeast (55). However, we observed no changes in Cdc42 expression or localization in cells lacking Bin3, including in lens cells where Bin3 loss caused a profound disruption in F-actin organization. The notion that the Bin3 and Cdc42 proteins may not interact constitutively in mammalian cells, as their fission yeast counterparts do, is not entirely unexpected given (a) the profound difference in the effects of deleting Cdc42 and Bin3 in the mouse, only the former of which yields an early embryonic lethal phenotype associated with gross structural abnormalities, and (b) the much greater degeneracy in Rho small GTPases and their regulators in mammals compared with yeast. Given evidence that Bin3 and its yeast homologues may share roles in stress signaling (5), possibly germane to cancer suppression, along with the involvement of Cdc42 in motility and perhaps cytokinesis (55), it is interesting that oncogenically transformed primary cells lacking Bin3 displayed an increased sensitivity to the antimitic agent benomyl as well as an increased motility in transwell migration assays. Thus, interactions of Bin3 with Bin1 or Cdc42 may be contingent on a cellular stress state, including oncogenic stress, the context of which may help define their possible roles in cell proliferation and motility.

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