Phosphatidylinositol 3-Kinase Is Necessary for Lens Fiber Cell Differentiation and Survival

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PURPOSE. To determine the mechanisms of action of phosphatidylinositol 3-kinase (PI3K) in lens cell differentiation and survival.

METHODS. Primary quail lens cell cultures were treated at different stages of differentiation with the PI3K inhibitor LY294002, and expression of survival proteins and differentiation markers were determined by immunoblot analysis. The connection between PI3K regulation of lens differentiation and actin cytoskeleton reorganization was examined by fluorescence-phalloidin staining and Rac activity assay. Survival in the absence of PI3K signaling was examined by TUNEL and DAPI staining. Phosphorylation of the PI3K effector glycogen synthase kinase-3 (GSK3) in the absence of PI3K signaling was induced with lithium chloride.

RESULTS. Exposure to LY294002 blocked lens epithelial cell differentiation initiation. This result was linked to attenuation of Rac activity and inhibition of actin filament reorganization from stress fibers to cortical fibers, which has been shown to signal lens differentiation initiation. The survival of lens epithelial cells in the absence of PI3K signaling correlated with the induction of numerous survival factors, including Bcl-2. In contrast, inhibition of PI3K signaling in differentiating lens fiber cells induced apoptosis by blocking inactivation of GSK3, showing that PI3K/GSK3 signaling has a protective role in the late stages of differentiation as nuclei and organelles are lost.

CONCLUSIONS. PI3K signaling regulates lens cell differentiation initiation through its ability to signal reorganization of the actin cytoskeleton from stress fibers to cortical fibers. In differentiating lens fiber cells, PI3K has a protective function, signaling survival through inactivation of its downstream effector GSK3. (Invest Ophthalmol Vis Sci. 2006;47:4490–4499) DOI: 10.1167/iovs.06-0401

Differentiation of lens epithelial cells into mature lens fiber cells involves the coordinated signaling of growth factor receptors and adhesion molecules and the reorganization of the actin cytoskeleton from stress fibers to cortical fibers. Actin stress fibers present in lens epithelial cells play a key role in maintaining an undifferentiated phenotype, and the disassembly of actin stress fibers initiates lens cell differentiation. As both a regulator of actin cytoskeletal organization and an effector molecule downstream of growth factor receptors and adhesion molecules, PI3K is perfectly poised to have a central role in the signaling events leading to lens cell differentiation. One mechanism by which PI3K may regulate lens cell differentiation is through its known function as an upstream activator of the small RhoGTPase Rac. Activation of Rac antagonizes Rho causing disassembly of actin stress fibers, a key inductive signal for lens cell differentiation. Therefore, PI3K, through its ability to activate Rac, may be essential for the disassembly of actin stress fibers and the consequential initiation of lens cell differentiation.

In addition to its role as a differentiation signal, PI3K also provides survival signaling in a number of diverse cell and tissue types, from neurons to metastatic cancers. PI3K may provide a survival signal in the developing lens by counteracting the apoptosis-related pathways that are necessary for the differentiation of lens epithelial cells into mature lens fiber cells. Initiation of lens cell differentiation requires activation of apoptosis-related Bcl-2 and caspase-dependent (ABC) pathways involving the activation of caspase 3-like proteases. Another set of apoptotic pathways involving proapoptotic molecules like caspase 6 are activated during terminal maturation of lens fiber cells. These pathways contribute to remodeling of the membrane cytoskeleton and the formation of an organelle-free zone (OFZ) in the center of the lens that is devoid of nuclei, mitochondria, endoplasmic reticulum, and Golgi. The apoptosis-related molecules involved in both ABC differentiation and terminal maturation must be balanced by the activation of cell survival mechanisms that prevent commitment to the cell death program. The survival pathways that protect lens fiber cells as they differentiate are not well understood. In the adult bovine lens, PI3K activity is high both in the lens epithelium and throughout most of the terminally differentiating lens fiber cell zones, where it may have a role in cell survival. PI3K/Akt signaling has been shown to protect cultured lens epithelial cells against staurosporine-induced apoptosis, yet the importance of PI3K as a survival factor in lens differentiation has not been established.

PI3K signaling results in the activation of its primary downstream effector Akt/PKB. Akt, a serine/threonine kinase, has several targets that promote survival. Phosphorylation of Bad by Akt allows the antiapoptotic proteins Bcl-2 and Bcl-X<sub>L</sub> to protect against mitochondria-induced apoptosis. Moreover, PI3K/Akt signaling has been shown to protect against apoptosis following cell death. In addition, glycogen synthase kinase-3 (GSK3) has been identified as an Akt substrate, where the phosphorylation by Akt inhibits GSK3 activity. Unregulated GSK3 activity is capable of inducing apoptosis in a variety of cell types, and its inhibition by Akt-mediated phosphorylation can promote cell survival.

We investigated the role of PI3K in signaling both differentiation and survival of lens fiber cells. Furthermore, we examined the potential roles of Rac and GSK3 as signaling molecules downstream of PI3K in a developmental system that requires apoptotic-related pathways for cellular differentiation.

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METHODS

Lens Microdissection

Lenses were isolated from embryonic day (E)10 chicken eggs (Truslow Farms, Chestertown, MD) and microdissected as previously described to yield four distinct regions of differentiation: the central anterior epithelium (CE), the equatorial epithelium (EQ), cortical fiber cells (FP), and the central fiber zone (FC).27

Preparation and Treatment of Primary Lens Cultures

Differentiating primary lens cell cultures were prepared as previously described.28 Briefly, lens cells were isolated from E10 quail lenses by trypsinization and agititation. Cells were plated on laminin and cultured in M199 with 10% FBS. For the inhibition of PI3K, cultures were treated with 25 μM LY29402 or 500 nM wortmannin (Biomol, Plymouth Meeting, PA). Lithium chloride (5 mM) was used to inhibit GSK3 (Sigma-Aldrich, St. Louis, MO). ERK activity was inhibited with 20 μM U0126 (Biomol). Control cultures were exposed to dimethyl sulfoxide (DMSO; Sigma-Aldrich), the solvent for the pharmacological agents used in these studies.

Protein Extraction

Microdissected lens fragments and culture samples were extracted in Triton/OG buffer (4.4 mM n-octyl β-D-glucopyranoside, 1% Triton X-100, 100 mM NaCl, 1 mM MgCl₂, 5 mM EDTA, 10 mM imidazole [pH 7.4]) containing 1 mM sodium vanadate, 0.2 mM H₂O₂, and protease inhibitor cocktail (Sigma-Aldrich). Protein concentrations were quantified using the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL).

Rac GTPase Activity Assay

Rac activity was assayed using the Rac GT-Pase activation kit from Pierce. The assay was performed as specified by the manufacturer. Briefly, 1 mg cell lysate was incubated with a GST-fusion peptide containing the Rac binding domain of PAK1 which only binds GTP-bound/active Rac. GST binding buffer (Swell Gel; Pierce) was used to bind the complex and retain it in the spin column. Complexes were eluted from the spin columns with Laemmli’s sample buffer and subjected to SDS-PAGE followed by immunoblot analysis with Rac1 antibody.

Immunoblotting and Antibodies

For all studies, 15 μg of protein extracts were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on precast Tris-glycine gels (Novex, San Diego, CA). Proteins were electrophoretically transferred onto membranes (Immobilon-P; Millipore, Bedford, MA). Bcl-2 (N-19), PI3K p110δ, GSK3 (Sigma-Aldrich, St. Louis, MO). ERK activity was inhibited with 20 μM U0126 (Biomol). Control cultures were exposed to dimethyl sulfoxide (DMSO; Sigma-Aldrich), the solvent for the pharmacological agents used in these studies.

PI3K in Lens Differentiation and Survival

Fluorescent Labeling of Actin

Lens cell cultures were fixed with 3.7% formaldehyde and permeabilized with 0.25% Triton X-100. Actin was labeled by incubating fixed cultures with AlexaFluor 488-conjugated phalloidin (Invitrogen, Eugene, OR). Fluorescence stained samples were then examined with a microscope (Eclipse 80i; Nikon), and images were acquired (Metamorph, ver. 6.2; Universal Imaging Corp., Downingtown, PA).

TUNEL Assay

Cultures were rinsed with PBS twice and fixed for 15 minutes in 3.7% formaldehyde diluted in PBS. A cell death detection kit (In Situ Cell Death Detection, TMR Red; Roche Molecular Biochemicals, Indianapolis, IN) was used to label the fragmented DNA of apoptotic cells. Nuclei of living and apoptotic cells were counterstained with DAPI (Invitrogen).

RESULTS

PI3K Regulation of Lens Cell Differentiation

We examined the functional role of PI3K in the developing lens using a cell culture system that closely mimics lens differentiation in vivo.26 Undifferentiated primary lens epithelial cells were isolated from embryonic quail and plated on a laminin substrate. Initially, these undifferentiated cells form colonies of well-spread epithelial cells (Fig. 1A, left). Within 5 days, these epithelial cells compacted into a cobblestone arrangement (Fig. 1A, center), the earliest morphologic indication for the initiation of lens cell differentiation.28 These cells then further differentiated in culture, resulting in the formation of multicellular, multilayered, lens-like structures known as lentoids (Fig. 1A, right). To examine whether PI3K has a requisite role in lens cell differentiation, undifferentiated lens epithelial cells were exposed to the specific PI3K inhibitor LY29402 for 72 hours beginning at day 2 in culture. This period of treatment encompasses the time during which these primary lens epithelial cells begin to differentiate. In contrast to control cultures, lens epithelial cells treated with LY29402 were unable to form a compacted epithelial monolayer. They remained as a well-spread epithelium, typical of undifferentiated lens cells throughout the culture period (Fig. 1B). The suppression of lens cell differentiation by treatment with LY29402 was paralleled at the biochemical level. At the initiation of differentiation, high levels of expression of the cell cycle inhibitor p27(KIP1) and the lens cell differentiation-specific proteins filensin, CP49, and δ-crystallin indicated that control lens cell cultures had both withdrawn from the cell cycle and begun their differentiation program (Fig. 1C, control). In the presence of LY29402, cultured lens cells continued to proliferate, as evidenced by their expression of PCNA and their failure to induce expression of the cell cycle inhibitor p27(KIP1; Fig. 1C). Previous studies from our laboratory and others have shown that cell cycle withdrawal is essential for initiation of the lens differentiation program.29–31 Therefore, the failure to induce p27(KIP1) expression is a good indication that LY29402-treated lens cells did not differentiate. Further supporting a role for PI3K in lens differentiation, we found that LY29402 suppressed expression of filensin and CP49, two differentiation-specific intermediate filament proteins of the lens (Fig. 1C). In contrast, LY29402 treatment caused a small but significant increase in the expression of δ-crystallin, in agreement with a previous report.26 Although δ-crystallin has been used as a differentiation marker, this result
suggests that the function of δ-crystallin in the absence of PI3K signaling is not linked to differentiation.

Control cultures maintained in normal media continued to differentiate and formed lens fiber cell-containing lentoid structures. In contrast, the continued suppression of PI3K activity with LY294002 resulted in the marked inhibition of lentoid formation (Fig. 1D). Most cells remained as an uncompacted monolayer typical of undifferentiated lens epithelial cells in culture. Together, these data demonstrate that PI3K signaling is necessary for both biochemical and morphologic differentiation of lens epithelial cells.

PI3K Regulation of Actin Organization

Reorganization of the actin cytoskeleton is critical for the differentiation of lens epithelial cells into lens fiber cells, both in culture and in vivo. As we have demonstrated, in undifferentiated lens epithelial cells, actin filaments are organized as actin stress fibers (Fig. 2A, time 0). Concurrent with the initiation of lens cell differentiation, actin stress fibers are disassembled, and actin filaments are reorganized as cortical fibers (Fig. 2A, 26 hours control). This disassembly of actin stress fibers provides a key inductive signal for lens cell differentiation.
One of the ways in which PI3K is likely to signal lens cell differentiation is through its ability to regulate actin cytoskeletal organization. We found that inhibition of PI3K by LY294002 in lens epithelial cells prevented this reorganization and actin remained as stress fibers. (B) Lens epithelial cells were allowed to compact, at which point actin has reorganized into cortical fibers. Cultures were labeled with fluorescent-conjugated phalloidin to visualize actin filaments. LY294002 treated cells were unable to maintain a cortical actin arrangement, and actin formed stress fibers characteristic of undifferentiated lens epithelial cells. (C) Activation of Rac, the small RhoGTPase responsible for signaling cortical actin organization, was significantly inhibited when lens cells were exposed to the PI3K inhibitor LY294002 for 28 hours ($P \leq 0.02$).

PI3K can signal formation of cortical actin structures is through activation of the small RhoGTPase Rac. Inhibition of PI3K in lens epithelial cells resulted in a significant decrease in Rac activity (Fig. 2C, $P \leq 0.02$) that was directly correlated with the presence of actin stress fibers and the inhibition of cortical actin fiber formation. These results demonstrate that PI3K is necessary for full activation of Rac in lens cells, a probable mechanism by which PI3K signals the disassembly of actin stress fibers and the reorganization of cortical actin fibers important during the initiation of lens cell differentiation.

**PI3K in the Survival of Lens Epithelial Cells**

PI3K is a survival signal in many cell types and it has a prosurvival function in lens epithelial cells treated with apoptogenic agents. To investigate whether PI3K signals cell survival of lens epithelial cells during development, primary lens epithelial cells were cultured in the presence of LY294002 for 72 hours...
One reason that LY294002 did not affect survival of lens epithelial cells may be that alternative survival pathways were activated in the absence of PI3K signaling. We investigated whether lens epithelial cells induce alternate survival signals in response to LY294002 treatment. Lens epithelial cells treated with LY294002 increased expression or activation of all survival signals that we examined (Fig. 3B). Expression of both the antipapoptotic protein Bcl-2 and the IAP survivin were induced in lens epithelial cells in response to LY294002 treatment. Bad phosphorylation is likely to be an important survival signal in the developing lens as it permits the survival functions of Bcl-2 and Bcl-x<sub>L</sub>. Surprisingly, although Ser<sup>136</sup> of Bad is a direct target of PI3K/Akt survival signaling in many cell types, phosphorylation of Bad on Ser<sup>136</sup> was actually increased by PI3K inhibition with LY294002 in lens epithelial cells. Other kinases, such as PAK1, p70S6K, or PKA, which can phosphorylate Bad on Ser<sup>136</sup> and other sites are probably responsible for Bad phosphorylation in the lens. Inhibition of PI3K did not affect the activation state of ERK (Fig. 3C). Lens epithelial cells appear to have an exceptional ability to turn on multiple survival pathways, probably reflecting the importance of these pathways in protecting the lens from outside stresses in the adult.

**Role of PI3K in Survival of Differentiating Lens Fiber Cells**

Apoptosis-related pathways are activated both for initiation of lens cell differentiation and for terminal differentiation of lens fiber cells. We postulated that differentiating lens fiber cells may have a specific need for a PI3K survival pathway to protect them from apoptotic-related pathways. To explore whether PI3K has a survival function in lens fiber cells, we cultured lens cells until differentiated lentoid structures had formed and then treated these cultures for 72 hours with LY294002. Exposure of late-stage cultures to LY294002 had a striking morphologic effect on the lens fiber cells that comprised the lentoid structures, inducing blebbing of the plasma membrane (Fig. 4A, phase, arrows) and nuclear condensation (Fig. 4A, DAPI, arrowheads), both characteristics of apoptotic cells. In addition, lens fiber cells treated with LY294002 had many TUNEL-positive nuclei (Fig. 4A, TUNEL), a marker of advanced-stage apoptosis. Immunoblot analysis for activated Akt (Akt P-Thr<sup>308</sup>) demonstrated that the induction of apoptosis in differentiating lens fiber cells after inhibition of PI3K correlated directly with the suppression of Akt activity. These data demonstrate that PI3K/Akt signaling is necessary for survival of lens fiber cells.

The differential survival response of lens epithelial and fiber cells to the loss of PI3K function suggested that the protective machinery was very different in undifferentiated and differentiated lens cells. Although many survival signals had higher basal levels of expression in lens fiber cells than in lens epithelial cells (data not shown), none of the Bcl-2 family or IAP survival signals that we examined in lens fiber cells were decreased below basal levels on treatment with LY294002 (Fig. 4B). These data suggest that another survival signal, other than Bcl-2 or IAPs, must be regulated by PI3K to signal survival in lens fiber cells.

**The Mechanism of PI3K/AKT Survival Signaling in Differentiating Lens Fiber Cells**

In addition to regulation of Bcl-2 and IAP family members, the PI3K/Akt pathway is responsible for phosphorylation and consequent inactivation of GSK3. Of note, we found that phosphorylation of GSK3 was the only survival signal we examined...
To examine whether PI3K signaled survival of lens fiber cells, we used lithium chloride, a well-known inhibitor of GSK3 activity. Exposure of cells to lithium chloride increases GSK3 phosphorylation and inhibits GSK3 activity independent of PI3K. We first tested whether lithium chloride would inhibit GSK3 in our lens cell cultures. Differentiated lens cell cultures were treated with increasing concentrations of lithium chloride for a 24-hour period. Lithium chloride treatment of lens cell cultures effectively increased phosphorylation of GSK3 and therefore inhibited its activity in a dose-dependent manner (Fig. 5B). Lentoid cultures were treated with LY294002 in the presence of 5 mM lithium chloride to determine whether increased phosphorylation of GSK3 would rescue the lens fiber cells from LY294002-induced apoptosis. We discovered that LY294002 induction of apoptosis in lens fiber cells, as determined by the TUNEL assay, was blocked in the presence of lithium chloride (Fig. 5C). The inhibition of GSK3 was sufficient to prevent apoptosis in the absence of PI3K activity. These data provide direct evidence that the PI3K survival mechanism in lens fiber cells involves PI3K phosphorylation of GSK3.

PI3K/GSK3 Survival Signaling Pathways in the In Vivo Embryonic Lens

Because our data indicate that PI3K/GSK3 signaling was essential to the survival of differentiating lens fiber cells in culture, we examined whether this survival mechanism was present in the in vivo developing lens. The embryonic lens provides a unique opportunity to study the role of PI3K signaling in cellular differentiation. At a single time point in development, multiple stages of differentiation are present, from undifferentiated epithelial cells to terminally differentiating fiber cells. The ability to separate these stages by microdissection techniques makes it possible to examine the dynamic processes that regulate lens cell differentiation in vivo. For this study, E10 chick lenses were separated into four regions: the undifferentiated central epithelium (EC); the equatorial epithelium (EQ) where lens cells initiate their differentiation; the cortical fiber zone (FP) where much of lens cell morphologic differentiation takes place; and the terminally differentiating core nuclear fiber region (FC; Fig. 6A). Because PI3K was necessary for the phosphorylation of GSK3 and lens fiber cell survival in vitro, we examined the expression of PI3K regulatory and catalytic subunits in microdissected E10 lens fractions. The most ubiquitously expressed PI3K regulatory subunit is p85. In the embryonic lens, p85 was highly expressed in lens epithelial cells and in the differentiating fiber cells of the cortical zone, with its greatest expression in the region of the equatorial epithelium (Fig. 6B). For PI3K to be active, the catalytic subunit p110α must also be present. In the embryonic lens expression of the p110α catalytic subunit of PI3K had an expression pattern similar to that of p85 (Fig. 6B). These results demonstrate that PI3K is expressed in vivo in a pattern consistent with its role in survival signaling in differentiating lens fiber cells.

To determine whether the PI3K/GSK3 survival pathway was induced in differentiating lens fiber cells in vivo, we examined the phosphorylated state of both isoforms of GSK3, GSK3α, and GSK3β, by immunoblot analysis of microdissected fractions of the E10 chick lens. We found that the greatest level of both GSK3α and GSK3β phosphorylation occurred in the cortical lens fiber cells (FP), the dynamic region of lens fiber cell differentiation (Fig. 6C). Together with our in vitro studies, these data are consistent with the phosphorylation of GSK3αβ through a PI3K pathway as an essential survival mechanism for
preventing apoptosis of lens fiber cells during their differentiation in vivo.

**DISCUSSION**

**A Differentiation-Specific Survival Signal**

PI3K is a potent survival signal in many tissues and cell types. Here, we demonstrate that PI3K provides an essential survival signal in differentiating lens fiber cells where apoptotic-related pathways are activated for normal differentiation processes. We also found that lens epithelial cells, unlike their differentiated counterparts, did not succumb to apoptotic cell death in the absence of PI3K signaling. However, inhibition of PI3K signaling in lens epithelial cells prevented their differentiation. We suggest that the activation of PI3K survival signals may serve as a checkpoint necessary for progression through the differentiation program (Fig. 7A). This checkpoint would prevent lens cells from activating apoptotic-related pathways for differentiation in the absence of PI3K for protection (Fig. 7C). Without PI3K survival signaling, the apoptotic-related mechanisms that execute terminal lens cell differentiation would lead to cell death.

PI3K has a similar role in the differentiation of keratinocytes. The terminal differentiation of keratinocytes shares many features with that of lens fiber cells, including the loss of organelles and nuclei through processes involving apoptotic-related pathways. Although caspases are activated for keratinocyte differentiation as in the lens, the premature death of differentiating keratinocytes is prevented by the activation of PI3K survival signals. Also paralleling our results with lens cells, undifferentiated proliferating keratinocytes do not need PI3K for survival, and inhibition of PI3K activity suppresses the expression of keratinocyte differentiation markers. Therefore, in both keratinocyte and lens cell differentiation, PI3K appears to be a checkpoint necessary for differentiation to progress. The few keratinocytes that initiate differentiation in the absence of PI3K signaling ultimately undergo apoptosis.
In our studies, we examined several potential targets of PI3K survival signaling in the lens including members of the Bcl-2 and IAP families. Surprisingly, none of the Bcl-2 family members or IAPs that we examined was suppressed in the presence of the PI3K inhibitor LY294002. In fact, the only survival signal that we observed to be decreased by PI3K inhibition was P-GSK3. We have discovered that GSK3 plays a central role in mediating the PI3K survival signal. GSK3 inhibition was able to rescue the apoptotic phenotype of lens fiber cells treated with LY294002, demonstrating that GSK3 is a principal downstream effector of the PI3K signaling pathway essential to lens fiber cell survival. Similar to our findings in lens fiber cells, the PI3K/Akt-mediated inhibition of GSK3 is an important survival signal in erythroblasts, and is suggested to have a role in the PI3K-mediated survival of differentiating keratinocytes. Our study is the first demonstration of a differential requirement for regulation of GSK3 as a cell survival signal during development (Fig. 7C).

**Potential Targets of the PI3K/GSK3 Survival Signal**

The exact mechanism for GSK3 induction of apoptosis has remained elusive, but one proposed mechanism that is intriguing for lens development involves the tumor-suppressor gene p53. GSK3 signaling has been shown to prevent degradation of p53, activate p53 through phosphorylation, and change p53 distribution. All these GSK3-mediated effects promote p53 induction of apoptosis. In the lens, p53 signals the loss of organelles and nuclei associated with terminal differentiation through its apoptotic-related functions. Nuclear degradation is significantly delayed in lenses of mice expressing a loss-of-function mutant p53, but overexpression of wild-type p53 induces death of lens fiber cells. This balance of p53 activity is also important in differentiating erythrocytes, another cell type that loses nuclei and organelles, where p53 promotes differentiation. The regulation of GSK3 by the PI3K pathway may be critical to controlling p53 localization and function. Whereas GSK3 may promote p53 function for lens cell differentiation, the PI3K survival pathway regulates GSK3 to prevent commitment to apoptotic cell death. GSK3 may also affect apoptosis through its role in the degradation of \(\beta\)-catenin. Active GSK3 forms a complex with other proteins that together phosphorylate \(\beta\)-catenin as a tar-
geiting mechanism for β-catenin degradation. Inhibition of
GSK3 allows β-catenin levels to accumulate in the cell for
multiple purposes. As a component of the canonical Wnt
pathway, β-catenin functions as a transcription factor where,
in conjunction with the Tcf/Lef complex, it promotes the
expression of proteins involved in numerous cellular processes
including survival. In the lens, β-catenin is translocated to the
nucleus of differentiating lens fiber cells, suggesting a possible
transcriptional role for β-catenin. In addition, β-catenin
serves as a scaffolding molecule that provides part of the
linkage between classic cadherins and the actin cytoskeleton
forming stable adherens junctions. These cell–cell cadherin-
based junctions also promote cell survival signals. During
lens cell differentiation, N-cadherin linkage to the cytoskeleton
increases—a probable function of increased N-cadherin-
catenin association. The regulation of GSK3 by the PI3K
signaling pathways is likely to be necessary to prevent the degra-
dation of β-catenin involved in both transcriptional and
adhesion processes that may promote lens cell survival.

Potential Upstream Inducers of PI3K Signaling
PI3K is an effector molecule of many receptor signaling mole-
cules, including integrins, cadherins, and growth factor recep-
tors. The adhesion receptors α6 integrin and N-cadherin are
among the signaling molecules that have been identified as
initiating PI3K signaling. N-cadherin function and PI3K activity
are all essential in lens cell differentiation. The requirement of α6 integrin and N-
cadherin for lens cell differentiation may be directly linked
with a role for these molecules in supporting lens fiber cell
survival through PI3K signaling. In addition to adhesion mole-
cules, numerous growth factors activate PI3K. IGF-1 induces
activation of PI3K in lens cells, and this pathway protects
lens epithelial cells against staurosporine-induced apoptosis.
FGF-2 also can activate PI3K signaling pathways, and in
lens cells FGF-2 increases IGF-1-mediated activation of PI3K.
Furthermore, growth factor receptors can cooperatively signal
with adhesion molecules to activate downstream signaling
pathways. For example, cross-talk between E-cadherin and
EGFR is important for PI3K survival signaling during keratino-
cyte differentiation. Future studies will be conducted to inves-
tigate whether growth factor receptors, in conjunction with
integrins and cadherins, are involved in the upstream activation of PI3K survival signaling in the developing lens.

Activation of Rac in PI3K Regulation of Lens Cell
Differentiation and Survival
PI3K signaling has profound effects on actin cytoskeletal organi-
ization through its role as a regulator of RhoGTPases, signal-
ing molecules that instruct actin organization. In this study in
lens cells, PI3K activated Rac, a RhoGTPase that signals disas-
sembly of actin stress fibers and formation of cortical actin
fibers. Loss of PI3K function attenuated Rac activation and
prevented both formation and maintenance of cortical actin
fibers. Disassembly of actin stress fibers is a critical step for the
initiation of lens differentiation. Because Rac can be inhibitory to Rho-mediated formation of actin stress fibers, PI3K activa-
tion of Rac in lens cells may signal the disassembly of actin stress fibers resulting in the initiation of differentiation (Fig.
7B). Reorganization of actin into cortical fibers as occurs in
differentiating lens cells is also probably signaled by PI3K
activation of Rac. Furthermore, we have shown that Rac activa-
tion is necessary for lens fiber cell survival, similar to PI3K.
Of note, however, the inhibition of GSK3 by lithium chloride
was sufficient to rescue lens fiber cells from LY294002-induced
apoptosis, suggesting that phospho-GSK3 and Rac may be part
of a common PI3K-dependent pathway necessary for lens fiber
cell survival.

We have identified PI3K as an important survival signal in
differentiating lens fiber cells. The functions of PI3K as a
differentiation-specific survival signal and in signaling activa-
tion of Rac play an important role in enabling differentiation of
lens fiber cells. These studies have further increased our un-
derstanding of the survival mechanisms that regulate apoptot-
ic-related pathways that are activated during normal cellular
differentiation, a phenomenon that occurs in many different
tissues.

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