

# The Nir proteins: From cellular functions to blindness

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### Introduction

Retinal degeneration, either acquired or inherited, is a major cause of visual impairment and blindness in humans. Inherited retinal degeneration comprises a large group of diseases that result in the loss of photoreceptor cells. To date, 131 retinal disease loci have been identified, and 76 of the genes at these loci have been isolated. Several of these genes were first considered candidates because of their chromosomal localization or homology to genes involved in retinal degeneration in other organisms. Our study is focused on a novel family of genes designated Nirs, which are candidates for inherited human retinal degeneration diseases.

### Nirs

The long term goal of our studies is to elucidate the cellular functions of Nirs and their putative role in inherited human retinal degeneration diseases. We apply both animal models and advanced molecular biology, cell biology, biochemistry, and imaging techniques in order to define their cellular functions, and to understand at the molecular and cellular levels their involvement in retinal degeneration and blindness.

Nirs (Nir1, Nir2 and Nir3) were first isolated as interacting proteins with the tyrosine kinase PYK2, using the yeast two-hybrid screen. They consist of several conserved structural domains, including an N-terminal phosphatidylinositol (PI)-transfer domain which specifically transfers PI and phosphatidylcholine between membrane bilayers, an acidic region that binds calcium, six hydrophobic stretches, and a C-terminal domain that binds the tyrosine kinase PYK2. They belong to a highly conserved family of proteins, which have been found in flies, fish, worms and mammals. Nevertheless, nothing is known on their cellular functions. Most of the studies to date have been focused on the *Drosophila* homologue *retinal degeneration B* (*rdgB*), a protein which is implicated in the visual transduction cascade in flies. *RdgB* mutant flies exhibit light-enhanced retinal degeneration and an abnormal electroretinogram. Genetic, biochemical and electrophysiological studies have suggested that *rdgB* is required for a proper termination of the light response and dark recovery of the photoreceptor cells, and that the PI-transfer domain is critical for these functions.

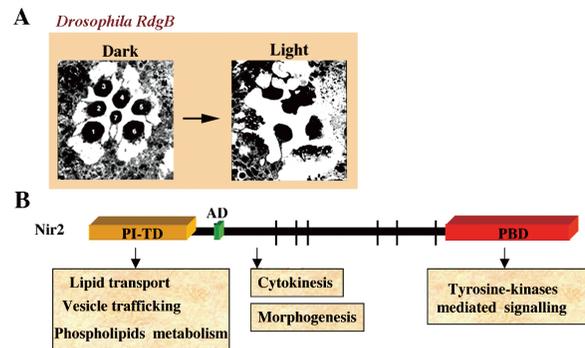


Fig. 1 A. Phenotype of the *Drosophila* *rdgB*. Shown are the six outer photoreceptor cells (R1-6) of *rdgB* mutant flies before (dark) and after light exposure (light). B. Structure and putative functions of the Nir2 protein.

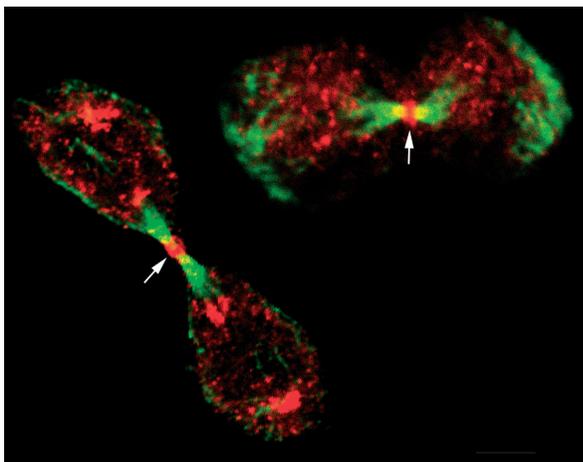
### Ongoing research activities

To assess the role of Nirs in retinal degeneration, we have recently established transgenic mice which express either wild-type Nir proteins or mutated Nirs in photoreceptor cells. We are currently characterizing their visual capabilities, sensitivity to light and the developmental and survival properties of their photoreceptors. In addition to this *in vivo* approach using animal models, we also apply cell and molecular biology based approaches to define their physiological functions. Although Nirs are highly expressed in the retina, they are also expressed in other tissues and distinctly found in diverse cell types. Therefore, we apply diverse cellular systems to assess several of their putative functions, including their involvement in polarity transport, lipid transport, cell morphogenesis, G protein-coupled receptor signaling, and PYK2-mediated signal transduction. Recently, we found that Nir2 plays important roles in **intracellular lipid transport**, **cell morphogenesis**, and **cytokinesis**. A specific mutation within the PI-transfer domain of Nir2 targets the protein to a new subcellular compartment and affects intracellular lipid transport. Thus, the PI-transfer domain is crucial for *rdgB*/Nir functions both in *Drosophila* photoreceptor cells and in mammalian cells, respectively. In addition to the role of Nirs in lipid transport, we have recently discovered two additional novel functions of Nirs: their regulatory role in cell

morphogenesis and in cytokinesis.

We found that Nir2 markedly affects cell morphology through a novel Rho-inhibitory domain (Rid) which resides in its N-terminal region. Rid specifically interacts with the inactive form of the Rho small GTPase, and inhibits Rho-mediated downstream signals. Our findings implicate Nir2 as a novel regulator of the small GTPase Rho in actin-cytoskeleton reorganization and cell morphogenesis.

Cytokinesis is the critical final stage of eukaryotic cell division, a process that requires coordinated movements of the plasma membrane and the cytoskeletal networks. This coordination is achieved by dynamic reorganization of the cytoskeleton at the cleavage furrow, and of the plasma membrane. We found that Nir2 is essential for normal cytokinesis, and we are currently investigating the molecular mechanisms underlying its function in this process.



**Fig. 2** Localization of Nir2 during cytokinesis. Shown are confocal images of dividing HeLa cells double immunostained for  $\beta$ -tubulin (green) and Nir2 (red). Nir2 is found in the middle of the microtubules bundles (arrow) at late telophase. Bar, 10 $\mu$ m.

### Perspective

Overall, we believe that Nirs coordinate membrane biogenesis and cytoskeleton rearrangements. Therefore, they have an important role in diverse cellular processes such as cytokinesis and cell morphogenesis. They may also be involved in photoreceptor cell physiology and retinal degeneration, as photoreceptor cells are polarized neurons which exhibit high membrane turnover due to the constant shedding of their outer segments. Therefore, photoreceptor cells are susceptible to an array of diseases that result in visual loss and blindness. Our challenge is to gain a better understanding at the molecular level of photoreceptor cell survival and degeneration, and to define genes/proteins which play a role in these processes. Nirs are only the beginning.

### Selected Publications

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- Lev, S.(2001) Molecular aspects of retinal degenerative diseases. *Cell. Mol. Neurobiology.* (in press).
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- Litvak, V., Shaul, D.Y., Tian, D., Shulewitz, M., and Lev, S. (2001) Regulated targeting of Nir2 to lipid droplets. (in preparation).

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