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Neuroglobin and Prion Cellular Localization: Investigation of a Potential Interaction

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Neuroglobin (Ngb) and the cellular prion protein (PrP^c), proteins of unknown function in the nervous system, are known to be expressed in the retina and have been observed in different rat retinal cells. The retina is the site of the highest concentration for Ngb, a heme protein of similar size and conformation to myoglobin. In this study, we demonstrated by immunohistochemical analysis of retinal colocalization of Ngb and PrP^c in the ganglion cell layer.

Considering for these two a common protective role in relation to oxidative stress and a possible transient contact during migration of PrP^c through the eye or upon neuronal degradation, we undertook *in vitro* studies of the interaction of the purified proteins. Mixing these two proteins leads to rapid aggregation, even at submicromolar concentrations. As observed with the use of dynamic light scattering, particles comprising both proteins evolve to hundreds of nanometers within several seconds, a first report showing that PrP^c is able to form aggregates without major structural changes. The main effect would then appear to be a protein–protein interaction specific to the surface charge of the Ngb protein with PrP^c N-terminal sequence. A dominant parameter is the solvent ionic force, which can significantly modify the final state of aggregation. PrP^c, normally anchored to the cell membrane, is toxic in the cytoplasm, where Ngb is present; this could suggest an Ngb function of scavenging proteins capable of forming deleterious aggregates considering a charge complementarity in the complex.

Keywords: neuroglobin; prion protein; retina; protein polymerization; scavenger

Introduction

Neuroglobin (Ngb) and the cellular prion protein (PrP^c) are both proteins of the nervous system of mammals whose functions have not yet been determined.[1–3] Both proteins are known to be expressed in the retina, the localization site of the highest concentration for Ngb.

Oxidative stress is involved in many ocular diseases, such as age-related macular degeneration, retinopathy of prematurity, retinal light damage, and cataract. Oxidative stress implies increased reactive oxygen species characterized by the production of free radicals, involved in cellular degeneration.

Ngb reversibly binds the ligands oxygen (O2), carbon monoxide (CO), and nitrogen oxide (NO); however, the external ligand is in competition with a histidine residue for binding to the ferrous iron. In addition, a pair of cysteines in the CD corner of Ngb may form a disulfide bond, which influences the overall oxygen affinity.[4] Ngb is thus a monomeric allosteric protein that may have a partner protein involving electron transfer to change the state of the iron or disulfide bond. Oxygen binding Ngb may have a protective role in response to neuronal ischemia

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Abbreviations used: Ngb, neuroglobin; Mb, myoglobin; PrP^c, cellular prion protein; GCL, ganglion cell layer; FTIR, Fourier-transform infrared; MAP2, microtubule-associated protein 2; PAF, paraformaldehyde; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate.

and can act as a scavenger of toxic species (nitrogen monoxide, hydrogen peroxide, etc.) and/or as scavenger and antioxidant in Alzheimer's disease.[5–7] Ngb is expressed at low levels (micromolar range) in the brain (cerebral cortex, hippocampus, thalamus, hypothalamus, and cerebellum) but at a much higher level

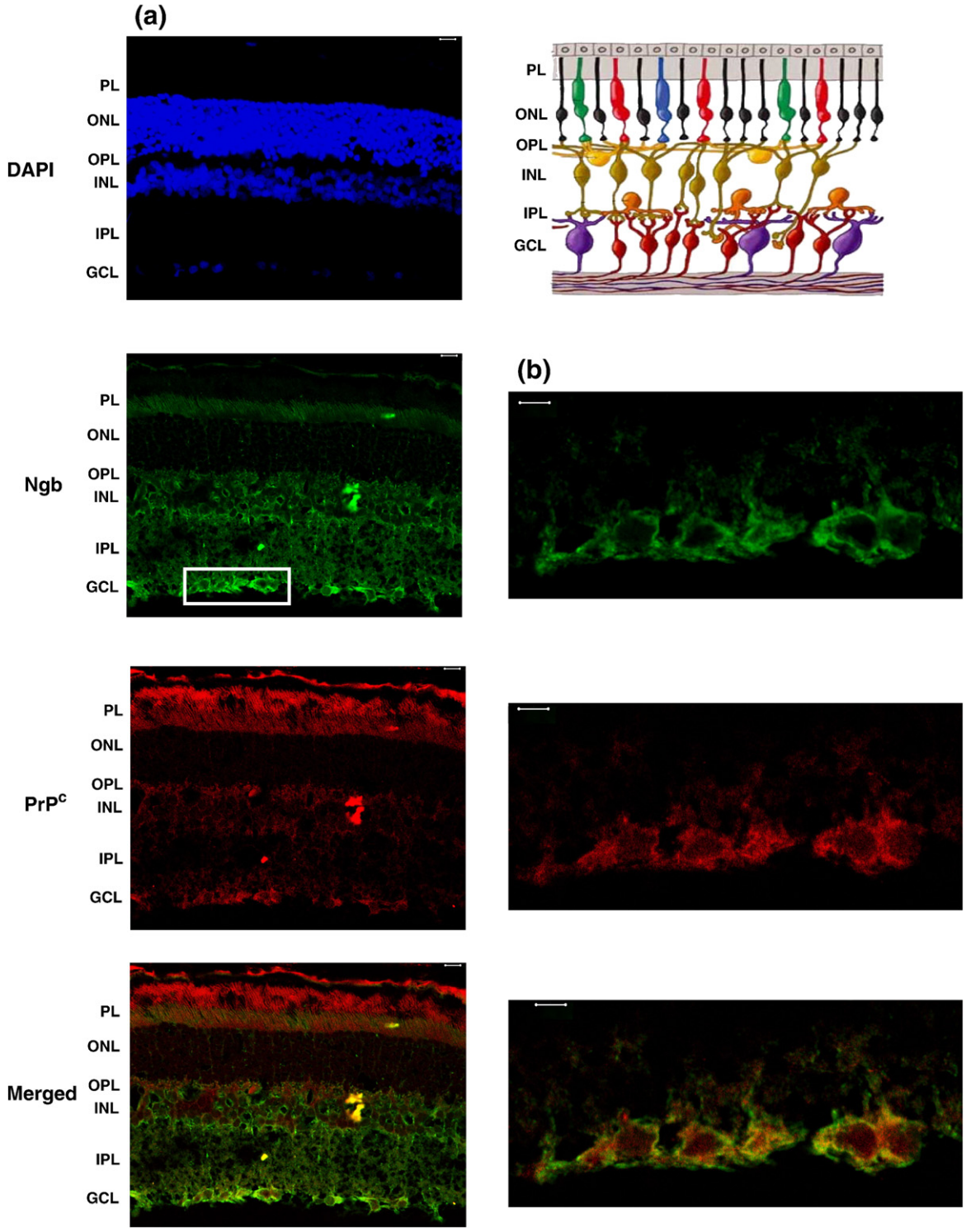

Fig. 1. (a) Ngb and PrP^c immunolocalizations were performed on cryostat rat retinal sections that were 10 μm thick. Confocal images illustrate the patterns of immunoreactivity for anti-Ngb rabbit polyclonal antibody and anti-PrP^c mouse monoclonal antibody. Confocal images of the retina doubly labeled for Ngb (green) and PrP^c (red) display a common cellular distribution in GCL illustrated by the significant colocalization of signals in the merged image. The scale bar represents 10 μm. (b) Ngb and PrP^c localization shows a more intense perinuclear staining pattern in the GCL. The scale bar represents 5 μm. Also shown (top) is the scheme of a vertical section of vertebrate retina:[22] PL, photoreceptor layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer.