Saturday, December 26, 2009

Frank Giblin and His Team Purify a Protein Found in the Eye

Recently Professor **Frank Giblin**, CBR member and director of the **Eye Research Institute**, and his team published a report in the journal **Protein Expression and Purification** (Volume 69, Pages 147-152, 2010) on the **Expression and Purification of His-Tagged Recombinant Mouse f-Crystallin**. Research Associate Mukoma Simpanya is the first author, and Research Technician Victor Leverenz is also a coauthor. Below is part of the introduction of the paper, with additional explanatory information in brackets. The research was supported by Giblin's grants from the **National Eye Institute**, one of the **National Institutes of Health**.

> "Our laboratory has found that chronic exposure of guinea pigs to UVA [**ultraviolet A**] light produces detrimental effects in the central region of the **lens** including increased **light scattering**, **disulfide crosslinking** of proteins and high molecular weight aggregate formation... We hypothesize that these effects are caused by absorption of UVA light in the guinea pig lens by **NADPH** [Nicotinamide adenine dinucleotide phosphate] bound to f-**crystallin** [a water-soluble structural protein found in the lens of the eye], leading to the generation of **reactive oxygen species** [small, highly reactive molecules that contain oxygen]…such as **superoxide anion** [O_2^-] and H_2O_2 [**Hydrogen peroxide**]. By absorbing UVA light in the lens, f-crystallin may act to protect the guinea pig **retina**…from UVA-induced damage. Indeed, retinas of guinea pigs that have been made **aphakic** [lacking a lens] have been reported to be damaged by…UVA light… Similar events may occur in the aging human lens where the binding of the UVA chromophore **kynurenine** [a metabolite of the amino acid **tryptophan** used in the production of **niacin**] to crystallins may lead to subsequent generation of damaging reactive oxygen species.
>
> The purpose of the present study was to employ **recombinant** methods [processes in which a molecule of **DNA** is broken and then joined to a different DNA molecule] to produce substantial amounts of f-crystallin which could later be used for **in vitro** investigation [in an articial environment like a test tube, as opposed to in a live animal] of mechanisms of UVA-induced damage in the lens. f-Crystallin has been **cloned** previously from **cDNA** libraries [DNA synthesized from a **messenger RNA** in a reaction catalyzed by the enzyme **reverse transcriptase**] of guinea pig lens epithelial cells…guinea pig lenses…guinea pig and mouse liver…and human liver…. Other investigators have found that attempts to express recombinant guinea pig f-crystallin in **Escherichia coli** [a **bacterium** often used when producing proteins using recombinant DNA methods] resulted in low yields since the expressed protein deposited mainly into **inclusion bodies** [aggregates of proteins] …. In the current study, **histagged** [an amino acid sequence in proteins that consists of at least six copies of the amino acid **histidine**, and are often used when producing genetically modified proteins] recombinant mouse f-crystallin was overexpressed in E. coli in substantial amounts … and the protein was purified to homogeneity."

SUMMARY
Professor Frank Giblin, CBR member and director of the Eye Research Institute, and his team recently published a report in the journal Protein Expression and Purification (Volume 69, Pages 147-152, 2010) on the Expression and Purification of His-Tagged Recombinant Mouse f-Crystallin.

Created by Brad Roth (roth@oakland.edu) on Saturday, December 26, 2009
Modified by Brad Roth (roth@oakland.edu) on Saturday, December 26, 2009
Article Start Date: Saturday, December 26, 2009