Investigating the genotype to phenotype link for a visual protein

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Introduction

As DNA sequencing becomes increasingly routine in clinics for diagnosis and prescribing treatment of diseases, the problem of accurately characterizing the effects of genetic variants is becoming ever more important. Classification standards, such as the American College of Medical and Genomics standard, have been proposed to classify genetic variants ranging from “pathogenic” to “benign”⁴. However, the effects for most variants are unknown and have thus been classified as “variants of unknown significance” (VUS). Even more poorly understood are the gene products that manifest from these variants and the mechanisms by which they carry out their effects.

In the visual system, genetic variation in the RHO gene which encodes for the visual pigment rhodopsin is the most common cause of retinitis pigmentosa (RP), a degenerative retinal disease. Over 150 different mutations in RHO have been identified⁵ and more are being discovered every year. However, many of these variants are VUS because our ability to understand their effects is being outpaced by the rate that new variants are being discovered.

To keep pace with the increasing number of VUS requires a systematic approach that can be easily scaled and is able to classify variants accurately, which is difficult to achieve with a light activated protein. Recent advances in sequencing technologies, however, have accelerated the development of deep scanning mutagenesis, a promising solution to this dilemma that offers the potential for rapid classification of the effects for thousands of variants in a single experiment.

In this study, we aim to develop and apply a deep scanning mutagenesis approach that combines fluorescence-activated cell sorting and deep sequencing to help classify rhodopsin variants and to determine the mechanisms by which these variants confer their effects.

A variant library for testing all possible missense variants within a functionally important region in rhodopsin

- The variant library comprises of 1,576 missense variants where each of the 104 sites in the target region was mutated to all 19 other amino acids.
- Extracellular loop 2 (EL2) is a major hotspot where RP mutations are known to cluster.
- Helix 5 (H5), Intraexcllular Loop 3 (ICL3), and Helix 6 (H6) undergo pronounced conformational shifts during receptor activation.
- ICL3 are within contact distance with downstream Gx G protein

Rhodopsin activation and protein abundance varies widely among variants

For most variants, sufficient receptor abundance is required to elicit detectable signaling

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Well-characterized misfolded variants are heterogeneously expressed on the cell surface

- P23H and V141del amino acid substitutions are known to cause rhodopsin to misfold while N200I is characterized to be superactive (Chen et al. in prep)

Rhodopsin activation and protein fluorescence

- Mislabeled proteins in yeast are known to form aggregates⁶
- Can we use differences in membrane heterogeneity to distinguish misfolded variants from those that fold but do not signal?⁷

Future directions

- Use the FACS-Seq approach to complete a deep mutational scan of the library presented here
- Image more variants to test whether differences in membrane heterogeneity can be used to distinguish misfolded variants from those that fold but do not signal

References