Editorial

Proteome of the degenerating retina

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In the last issue of the *Journal of Proteome Research*, Ly *et al.* (1) communicate on their kinetic analysis of the proteome of the *rd10* mouse, a model of inherited retinal blindness. Why this study is so important for our understanding of retinal diseases?

Inherited retinal degenerations are devastating diseases that affect more than one million people in the world. The genetic causes have been extensively studied and demonstrate their extreme heterogeneity as illustrated the large number of genes identified that mutations cause either autosomal recessive, autosomal dominant, X-linked, mitochondrial and even digenic diseases (2). The most prevalent form of these diseases, retinitis pigmentosa can arise from mutations in any of 58 genes known to date. The normal function of these genes is widely variable but their list is significantly enriched in proteins acting in the phototransduction cascade. The major clinical hallmark of retinitis pigmentosa is that it progresses through two temporally distinctive steps of photoreceptor loss. Rod photoreceptors degeneration leads to night blindness, a mild condition that is equivalent to another retinal dystrophy, congenital stationary night blindness (CSNB) (3). What makes retinitis pigmentosa so dreadful is the fact that contrarily to CSNB, the mutations that cause the disease can not only impede with the phototransduction cascade but lead to the death of rods by apoptosis (4). The loss of rods initiates a cascade of cone dysfunction followed by their degeneration leading to blindness in the absence of any treatment currently available. Because of the essential role of cones for color vision, but also for all visual functions in daylight environment, the mechanisms of secondary cone degeneration have been explored in great details which highlight the role of glucose metabolism and oxidative stress (5-8). In the absence of rods, the cone outer segments where are located the opsin molecules shorten due to a deficit in sugar metabolism resulting from the loss of expression of rod-derived cone viability factor (RdCVF) (5,9-11). Another consequence of rod-loss is an increase in oxygen tension in the retina that damages the cones, leading to their death later in life (12). This second phenomenon results from the fact that choroidal circulation, what brings oxygen and nutrients to photoreceptors through the retinal pigmented epithelium (RPE), is not regulated by photoreceptor needs. Since rods represent 95% of all photoreceptors in most mammal species, their degeneration results in retinal hyperoxia. It should be noticed that metabolic and redox deficits involved in secondary cone degeneration can be prevented by the two products of the nucleoredoxin like 1 gene (*NXNL1*), the trophic factor RdCVF and the thioredoxin enzyme RdCVFL (13).

Since blindness in patients suffering from retinitis pigmentosa is caused by non-cell autonomous degeneration of cones that results from cell autonomous degeneration of rods, preventing rod death would indirectly prevent central vision loss. The physiopathology of retinitis pigmentosa has benefited from spontaneous rodent models, especially the *rd1* mouse (14). The *rd1* mouse carries a recessive mutation in the *Pde6b* gene that encodes for the beta—subunit of the rod-specific phosphodiesterase that hydrolyses cyclic GMP (cGMP) in GMP upon light stimulation. This genetic defect leads to a non-physiological increase in cGMP concentration in rods that triggers somehow apoptosis. Photoreceptors maturate after birth in the mouse retina. Elevated cGMP is detected by post-natal (PN) day 8, and by PN21, all rods are lost. The model was originally used to identify gene with rod-specific expression by differential display of mRNA, but attempts to reverse the visual phenotype of the mouse was only successfully achieved by

using corrective gene therapy approaches that reintroduce a copy of the normal *Pde6b* cDNA (15). It was proposed that multiple pathways are activated during rod apoptosis in the *rd1* mouse and consequently targeting pharmacologically only one of these pathways is rather inefficient (16). This is the rational that led Ly *et al*. to study the kinetics profile of the proteome of the *rd10* mouse (1). The *rd10* carries a recessive mutation in the *Pde6b* gene, as the *rd1* mouse, but with a missense mutation in exon 13 of *Pde6b* gene, an allele that has some residual phosphodiesterase activity contrarily to the former which carries a null allele (17). As a consequence, rod degeneration is slower in the *rd10* retina and light responses can be recorded even one month after birth (18).

The retina is the only part of the brain where neurons are ordered in regular layers in which cells can be identified easily on sections though their position. In addition, the retina is dissected very efficiently and precisely because the outer retina that is composed of photoreceptors, rods and cones, adheres to the RPE through their outer segments, a link that can be disrupted to separate the retina while preserving its integrity. Under standard procedures all the layers from the whole surface of the retina of a wild-type mouse are evenly represented in each specimen. This allows accurate differential expression analysis. The transcriptome of mouse models of retinitis pigmentosa was first studied and revealed biologically significant changes in gene expression in rods and later in cones of the *rd1* retina (19,20). All retinal cell types respond during the process of retinal degeneration, while genes expressed specifically by photoreceptors (photoreceptor genes) have a reduced expression due to the loss of these cells, an injury response was observed from cells located in the inner retinal among which some endogenous neuroprotective genes. While being informative, the loss of expression of photoreceptor genes is indicative of the timing of the degenerative process, but it does not reveal the underlying mechanisms. Because of the chain reaction of the genomic response to photoreceptor degeneration, it is not possible to distinguish within the data, changes that are regulators of the apoptosis cascade from secondary events. One approach to the question would be to perform a kinetics analysis of the transcriptome of retina while degenerating in order to identify earliest events, then to inactivate these key regulator genes or to silence them and to validate their role in the death of photoreceptors. While technically feasible, this demonstration is still lacking. One could argue that following the elevation of cGMP, rod degeneration is initiated at the levels of the proteins. Transcriptomic analysis is best suited to analysis developmental processes where the organ is constructed, but proteomics is a better option to study degenerative processes, for organ deconstruction. Ly *et al*. applied this kinetic approach to study the proteome of the retina of the *rd10* mouse during rod degenerative process. The proteome of the end stage rod degeneration of the *rd1* retina was previously reported (21). While the sensitivity of proteomics is still lagging behind that of transcriptomics, it is in constant progression over the last decade. Using filter-associated sample preparation protocol and a liquid chromatographic separation of the tryptic peptides followed by MS/MS analysis on a LC-MS/MS analysis on LTQ OrbitrapXL Ly *et al*. identified a total of 2,885 different proteins, among which 2,620 were quantified (1). The data demonstrate that the approach paid off: at PN14, before any sign of rod degeneration can be observed in the *rd10* retina, only two proteins were found to be significantly differentially expressed between the *rd10* and the wild-type mouse. The reduced expression of PDE6G, a protein that is part of the phosphodiesterase complex is maybe a consequence of the PDE6B mutation rather than a consequence of the degenerative process. The authors concluded that the proteome does not reveal sign of degeneration at this stage. This is not the situation at PN28, when a total of 1,359 significant differences in protein expression have been identified between the two genotypes: the process is well advanced at this stage. The authors then focused their attention on the data at PN21, when only 57 significant changes were observed. The existence of 10 proteins expressed in photoreceptors with reduced expression at PN21 in that list (SAG, ABCA4, PROM1, CNGB1, BBS4, CNGA1, RGS9BP, PRPH2, ROM1, PDE6A) in addition to PDE6G reduced at PN14 and PEDE6B, the mutated protein, indicates that the process of degeneration is initiated in a significant proportion of rods by PN21. Mutations in the genes encoding for all of these proteins are causing retinitis pigmentosa or/and other inherited retinal dystrophies (2). The authors then used bioinformatics methods to cluster the data and to draw an interaction map that points on the implication of STAT signaling in rod degeneration, an observation that was validated in additional experiments. One could argue that interaction maps are based on prior knowledge and that important data may be outside the scope of such analysis, but the data now exist. Genes that are well studied exert an attraction in such analysis that is independent of their real implication

in the biological process under scrutiny. Given the fact the increased STAT1 immunoreactivity matches that of a Muller glial cell marker, the induction of this signaling might still belong to the injury response to rod cell death. Due to the increasing power of proteomic instruments, it is very likely the question of the mechanisms of rod degeneration in mouse models of retinitis pigmentosa will be resolved in the near future. Post-translational modifications of proteins such as phosphorylations are analyzed more accurately by current instruments. Techniques to capture oxidation status of proteins, known as redox proteomics, are emerging (22).

What else could accelerate such resolution in addition to technical improvements in accordance to the objective of the group of Stefanie Hauck? Perhaps, a more precise definition of the earliest proteomic events: PN21 might not be the perfect timing. Since some of the events observed by Ly *et al*. are not taking place in the cells that die, it could be essential to separate the outer retina that is composed of 97% rods to get an even precise view. This can be done by sectioning the retina with a vibratome or even by other techniques with higher resolution (23,24). Anyway, the study of Ly *et al*. provides a set of essential data and the study is a key step toward this scientific objective which brings a medical hope.

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Footnote

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References

1. Ly A, Merl-Pham J, Priller M, et al. Proteomic Profiling Suggests Central Role Of STAT Signaling during Retinal Degeneration in the rd10 Mouse Model. J Proteome Res 2016;15:1350-9.
2. RetNet: Summaries of Genes and Loci Causing Retinal Diseases. Available online: https://sph.uth.edu/retnet/sum-dis.htm
3. Zeitz C, Robson AG, Audo I. Congenital stationary night blindness: an analysis and update of genotype-phenotype correlations and pathogenic mechanisms. Prog Retin Eye Res 2015;45:58-110.
4. Doonan F, Groeger G, Cotter TG. Preventing retinal apoptosis--is there a common therapeutic theme? Exp Cell Res 2012;318:1278-84.
5. Aït-Ali N, Fridlich R, Millet-Puel G, et al. Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. Cell 2015;161:817-32.
6. Komeima K, Rogers BS, Lu L, et al. Antioxidants reduce cone cell death in a model of retinitis pigmentosa. Proc Natl Acad Sci U S A 2006;103:11300-5.
7. Punzo C, Kornacker K, Cepko CL. Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. Nat Neurosci 2009;12:44-52.
8. Wang W, Lee SJ, Scott PA, et al. Two-Step Reactivation of Dormant Cones in Retinitis Pigmentosa. Cell Rep 2016;15:372-85.
9. Busskamp V, Duebel J, Balya D, et al. Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. Science 2010;329:413-7.
10. Cepko C, Punzo C. Cell metabolism: Sugar for sight. Nature 2015;522:428-9.
11. Krol J, Roska B. Rods Feed Cones to Keep them Alive. Cell 2015;161:706-8.
12. Stone J, Maslim J, Valter-Kocsi K, et al. Mechanisms of photoreceptor death and survival in mammalian retina. Prog Retin Eye Res 1999;18:689-735.
13. Mei X, Chaffiol A, Kole C, et al. The Thioredoxin Encoded by the Rod-Derived Cone Viability Factor Gene Protects Cone Photoreceptors Against Oxidative Stress. Antioxid Redox Signal 2016;24:909-23.
14. Farber D, Flannery J, Bowes-Rickman C. The Rd mouse story — 70 years of research on an animal-model of inherited retinal degeneration. Progress in Retinal and Eye Research 1994;13:31-64.
15. Bennett J, Tanabe T, Sun D, et al. Photoreceptor cell rescue in retinal degeneration (rd) mice by in vivo gene therapy. Nat Med 1996;2:649-54.
16. Doonan F, Donovan M, Cotter TG. Activation of multiple pathways during photoreceptor apoptosis in the rd mouse. Invest Ophthalmol Vis Sci 2005;46:3530-8.

17. Chang B, Hawes NL, Hurd RE, et al. A new mouse retinal degeneration (rd10) caused by a missense mutation in exon 13 of the beta-subunit of rod phosphodiesterase gene. Invest Ophthalmol Vis Sci 2000;41:S533.
18. Byrne LC, Dalkara D, Luna G, et al. Viral-mediated RdCVF and RdCVFL expression protects cone and rod photoreceptors in retinal degeneration. J Clin Invest 2015;125:105-16.
19. Hackam AS, Strom R, Liu D, et al. Identification of gene expression changes associated with the progression of retinal degeneration in the rd1 mouse. Invest Ophthalmol Vis Sci 2004;45:2929-42.
20. Punzo C, Cepko C. Cellular responses to photoreceptor death in the rd1 mouse model of retinal degeneration. Invest Ophthalmol Vis Sci 2007;48:849-57.
21. Cavusoglu N, Thierse D, Mohand-Saïd S, et al. Differential proteomic analysis of the mouse retina: the induction of crystallin proteins by retinal degeneration in the rd1 mouse. Mol Cell Proteomics 2003;2:494-505.
22. Perluigi M, Swomley AM, Butterfield DA. Redox proteomics and the dynamic molecular landscape of the aging brain. Ageing Res Rev 2014;13:75-89.
23. Reidel B, Thompson JW, Farsiu S, et al. Proteomic profiling of a layered tissue reveals unique glycolytic specializations of photoreceptor cells. Mol Cell Proteomics 2011;10:M110.002469.
24. Clérin E, Yang Y, Forster V, et al. Vibratome sectioning mouse retina to prepare photoreceptor cultures. J Vis Exp 2014;(94).

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