Focus on Molecules: Neural retina leucine zipper (NRL)

Hari Jayaram [a,b,*], Peng-Tee Khaw [a,b], Robert E. MacLaren [b,c], G. Astrid Limb [a]

[a] Division of Ocular Biology and Therapeutics, UCL Institute of Ophthalmology, London, UK
[b] NIHR Biomedical Research Centre for Ophthalmology, UCL Institute of Ophthalmology & Moorfields Eye Hospital, London, UK
[c] Nuffield Laboratory of Ophthalmology, University of Oxford, UK

1. Structure

The protein NRL (neural retina leucine zipper) (Uniprot Accession #P54845) is a basic motif-leucine zipper (bZIP) transcription factor of the *Maf* sub-family, involved in cellular differentiation and gene regulation, that has an essential intrinsic role in the development of rod photoreceptors (Mears et al., 2001 (cited within Nichols et al., 2010)). The gene is located on human chromosome 14q between bands 11.1 to 11.2 and encodes for a 237 amino acid protein with a molecular weight of 26 kDa that is located within the cellular nucleus. The leucine zipper domain is located between positions 187–208 (Fig. 1), with characteristic intertwined alpha helices forming a coil leading to a bifurcation at the DNA binding sites. The NRL protein is able to dimerise, forming both homo- and heterodimers. The dimerisation composition influences DNA binding, with each dimer subunit independently able to recognise one half of the recognition sequence, leading to increased binding specificity and control of transcription. The additional DNA binding site in the NRL protein is provided by a basic domain located between positions 159–185 (Fig. 1A) on the amino terminal side of the domain.

2. Function

NRL is a transcription factor which regulates the expression of rod photoreceptor specific genes including rhodopsin. It binds to a cis-regulatory element (NRL response element, NRE) located within the proximal rhodopsin promoter region to enhance transcription. The NRL protein undergoes post translational modification via phosphorylation by mitogen-activated protein kinases. Six isoforms have been observed in the nuclei of rod but not cone photoreceptor nuclei *in vivo* (Swain et al., 2001).

In vitro studies have shown that the leucine zipper domain of *NRL* physically interacts with the homeobox protein CRX (also critical for photoreceptor development), and demonstrates synergy in enhancing the activity of the rhodopsin promoter. NRL also binds to an element within the Nr2e3 promoter sequence and enhances its activity synergistically with *CRX* to suppress cone differentiation and promote that of rod photoreceptors. Deletion of *NRL* in mice results in a retina with no rods but with a more cone-like nature (Mears et al., 2001 (cited within Nichols et al., 2010)). Within the retina, NRL is expressed in rods (Fig. 1B) but can also be identified within cells in the pineal gland (Swain et al., 2001).

In keeping with its role in the regulation of rhodopsin expression, *NRL* expression precedes that of rhodopsin during rod photoreceptor development in mammals. Transplantation of retinal progenitor populations in mice has demonstrated that successfully integrated rod photoreceptors may be derived from committed post-mitotic precursors expressing *NRL*, and not from proliferating progenitor/stem cells (MacLaren et al., 2006).

3. Disease involvement

The influence of *NRL* upon rhodopsin expression has led to the identification of mutations within the gene being associated with forms of retinal degeneration such as retinitis pigmentosa (RP). Genetic studies of patients affected by autosomal dominant RP have shown several missense mutations affecting residues including 50, 51 and 122 of the NRL protein. Subsequent *in vitro* work has suggested that the mutations seen in dominant forms of RP are due to an *NRL* protein with reduced phosphorylation and abnormally enhanced transcriptional activation of the rhodopsin promoter (Kanda et al., 2007). More recent work, however, has shown that some *CRX* mutations causing retinal degeneration reduce NRL activity through aberrant binding, which might support a separate mechanism of retinal degeneration in humans through *NRL* haploinsufficiency which is not seen in mice (Nichols et al., 2010).

* Corresponding author. Division of Ocular Biology & Therapeutics, UCL Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK. Tel.: +44 20 7608 6927; fax: +44 20 7608 6887.

E-mail address: h.jayaram@ucl.ac.uk (H. Jayaram).
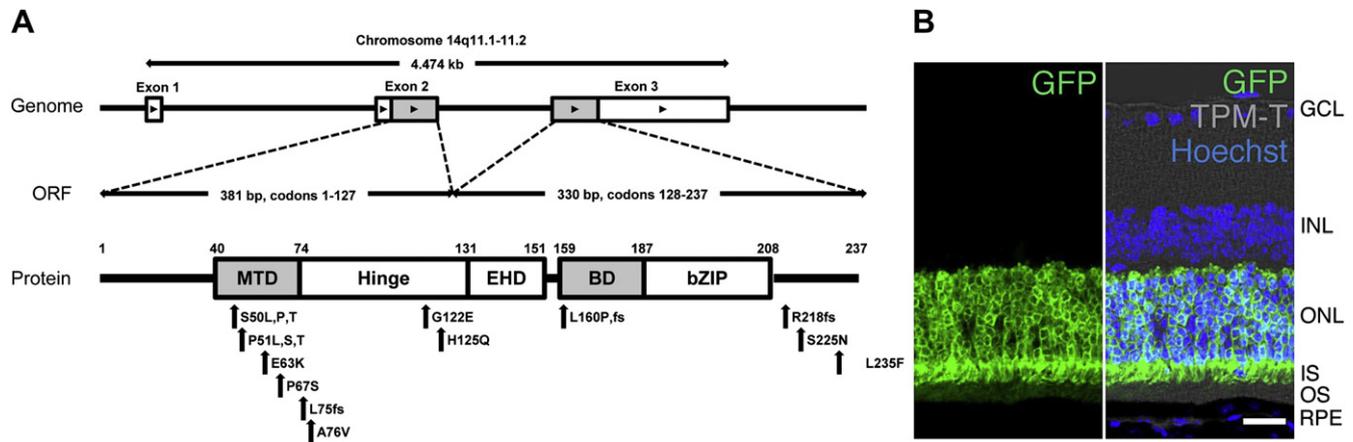
Fig. 1. A. Schematic diagram of the human *NRL* gene and protein. The *NRL* gene encodes 3 exons, the first of which is untranslated due to the absence of an ATG initiation codon. Annotation of the protein shows the minimal transactivation domain (MTD), hinge region, extended homology domain (EHD), basic domain (BD) and leucine zipper (bZIP). The locations of altered amino acid residues observed in patients with retinopathies are shown with arrows. (NRL gene and protein diagram adapted from Nishiguchi et al., 2004 (cited within Kanda et al., 2007).) B. Confocal projection image of a section of adult retina from the *Nrl.gfp*^{+/+} mouse demonstrating expression of *NRL* within the outer nuclear layer and rod inner segments. (RPE, OS, IS, ONL, INL and GCL refer to the retinal pigment epithelium, outer segments, inner segments, outer nuclear layer, inner nuclear layer and ganglion cell layer respectively. TPM-T = Nomarski image; scale bar = 20 μm).

Mutations within the *NRL* gene are also associated with recessively inherited photoreceptor degeneration. These include a leucine to proline substitution at residue 160, which results in an abnormal protein without a bZIP domain that is unable to bind to the NRE and showed reduced transcriptional activation of the rhodopsin promoter (Kanda et al., 2007). Recessive disease in some patients is also associated with the observation of enhanced S-cone function, as described in the *NRL* knockout mouse. The identification of loss of NRL function (either through haploinsufficiency or recessive disease) as a mechanism is critical for potential gene replacement therapy. This is because the *NRL* coding sequence is small at about 1.25 kb and therefore ideally suited for gene replacement using adeno-associated viral vectors which are being tested in a number of clinical trials.

4. Future studies

Investigation into the post translation modifications of NRL by phosphorylation and sumoylation may provide further insights into the regulatory networks controlling rod photoreceptor development. The role of proteins such as NRL in circadian rhythms, due to its identification in the pineal gland, may elucidate further physiological pathways. It is likely that *NRL* will provide a focus for emerging translational strategies to improve retinal function involving gene therapy and stem cells. Single gene mutations affecting *NRL*, such as those seen in patients with retinal degenerations such as RP, are a potential and realistic target for future gene therapy studies. Furthermore, the introduction of the concept of an optimal ontogenetic stage for photoreceptor precursor transplantation in rodents (MacLaren et al., 2006) served as a paradigm shift for research into stem cell transplantation. Using *NRL* as

a marker of committed rod photoreceptor precursors *in vivo* may facilitate the localisation and functional assessment of transplanted cells, using established methods such as focal electroretinography or by the development of implantable devices to measure the electrophysiological response to individual cells. Further work is required to translate current knowledge regarding *NRL* into therapeutic strategies, including the role of *NRL* in the differentiation of human adult-derived retinal stem cells and from other sources such as induced pluripotent stem cell lines.

References

Kanda, A., Friedman, J.S., Nishiguchi, K.M., Swaroop, A., 2007. Retinopathy mutations in the bZIP protein NRL alter phosphorylation and transcriptional activity. Hum. Mutat. 28, 589–598.

MacLaren, R.E., Pearson, R.A., MacNeil, A., Douglas, R.H., Salt, T.E., Akimoto, M., Swaroop, A., Sowden, J.C., Ali, R.R., 2006. Retinal repair by transplantation of photoreceptor precursors. Nature 444, 203–207.

Nichols 2nd, L.L., Alur, R.P., Boobalan, E., Sergeev, Y.V., Caruso, R.C., Stone, E.M., Swaroop, A., Johnson, M.A., Brooks, B.P., 2010. Two novel CRX mutant proteins causing autosomal dominant Leber congenital amaurosis interact differently with NRL. Hum. Mutat. 31, E1472–E1483.

Swain, P.K., Hicks, D., Mears, A.J., Apel, I.J., Smith, J.E., John, S.K., Hendrickson, A., Milam, A.H., Swaroop, A., 2001. Multiple phosphorylated isoforms of NRL are expressed in rod photoreceptors. J. Biol. Chem. 276, 36824–36830.