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Series

A cross-eyed geneticist's view

I. Making sense of the lamin B receptor, a chimeric protein

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The vertebrate inner nuclear membrane protein, lamin B receptor, has an N-terminal ∼200 residue nucleoplasmic domain (NTD), and a ∼420 residue C-terminal domain (CTD) that anchors the NTD to the INM. Chen *et al* (2016) showed the NTD interacts with Xist long noncoding RNA to effect X chromosome inactivation in female mammals. Tsai *et al* (2016) showed the CTD has sterol reductase activity that is essential for viability. And Nikolakaki *et al* (2017) proposed a model to interconnect these disparate functions of this chimeric protein. It amuses me now to think back to 24 years ago, when I was concerned that these domains might have come together in a cloning artifact.

Keywords. Cross-eyed geneticist's view; lamin B receptor; chimeric protein

The lamin B receptor (LBR) is the best studied vertebrate inner nuclear membrane (INM) protein. Its N-terminal ∼200 residues protrude into the nucleoplasm, where they bind to B-type lamins and heterochromatin, and serve as a substrate for the CDK1, SRPK1 and Akt kinases. In contrast, the ∼420 residue C-terminal domain (CTD) is hydrophobic, forms eight membrane-spanning segments, possesses sterol C-14 reductase activity, and anchors the nucleoplasmic domain to the inner nuclear membrane. Mutations in LBR are associated with two rare human diseases, Pelger-Huët anomaly and Greenberg skeletal dysplasia (Turner and Schlieker 2016). Pelger-Huët anomaly is a relatively benign autosomal dominant disorder characterized by abnormal hypolobulation of granulocyte nuclei. A granulocyte is a type of white blood cell. Greenberg skeletal dysplasia (also known as hydrops-ectopic calcification-moth-eaten or HEM skeletal dysplasia) is an autosomal recessive perinatally lethal disease characterized by abnormal bone development with abnormal calcification (and a 'moth-eaten' appearance) and excessive fluid accumulation (fetal hydrops). Some LBR mutations found in Pelger-Huët individuals in the heterozygous state have also been found in HEM/Greenberg dysplasia fetuses, and Pelger-Huët anomaly has been observed in Greenberg skeletal dysplasia parents. Human and mouse genomes contain a second locus, TM7SF2, that encodes a 418 residue protein of the endoplasmic reticulum (ER) called SR-1, with 58% identity with the LBR CTD and possessing sterol C-14 reductase activity. Why do our genomes encode two proteins with sterol C-14 reductase activity? We do not as yet have an answer. But I take proprietary pride in the question, because the first hint of LBR's sterol reductase function came from my laboratory (Papavinasasundaram and Kasbekar 1994), and was soon confirmed by us and others (Silve *et al*. 1998; Prakash *et al*. 1999). My subsequent research interests veered off into other areas, but periodically I look up progress in LBR research, much as one might keep tabs on an old flame.

Three recent papers show this old flame is doing quite well. Chen *et al*. (2016) found that LBR's N-terminal domain interacts with the Xist long noncoding RNA in female mammals to spool in one of the two X chromosomes to the nuclear lamina and effect X chromosome inactivation (XCI), thus balancing gene dosage with male XY cells. Tsai *et al*. (2016) showed that the sterol reductase activity of LBR is essential for viability under sterol-restrictive growth conditions, despite the presence of TM7SF2. This challenges the earlier assumption that LBR provided at most redundant or cell-type-/developmental-stage-specific sterol biogenesis activity. And Nikolakaki *et al*. (2017) reviewed the current knowledge of the interplay between the structure, function and localization of LBR, and proposed a model to interconnect the disparate functions of the N- and C-terminal domains of this chimeric protein.

Chen *et al*. (2016) measured the expression of five X chromosome and two autosomal genes by single-molecule RNA fluorescence *in situ* hybridization (smFISH) in a male

mouse embryonic stem (ES) cell line containing a doxycycline-inducible Xist expressed from its endogenous locus. The X-chromosome transcripts, but not autosomal transcripts, were lost from the male ES cells after doxycycline-dependent Xist induction. One would expect only a 50% loss in a female system which still retains one active X. The silencing required the interaction between the Xist lnc RNA's LBR-binding domain (LBD) and LBR's arginine-serine (RS) motif. Deletion of the RS motif in a ΔRS-LBR (deleted for amino acids 71–90) abolished Xist binding and showed a defect of the X-linked gene silencing, and had no effect on the autosomal genes. Similar silencing defects were seen upon LBR knockdown or knockout in differentiating female ES cells. Xist binding and XCI was not affected by deletion of seven of the eight transmembrane domains (amino acid residues 237–615) in ΔTM-LBR, and both ΔRS-LBR and ΔTM-LBR localized properly in the nuclear envelope. XCI was not affected by knockdown of additional components of the nuclear lamina, such as Lamin B1 or Emerin. Interestingly, fusion of three copies of the viral BoxB RNA aptamer to the 3′ end of Xist RNA (Xist-BoxB, the BoxB aptamer binds tightly to the viral λN coat protein) and expression of the ΔRS-LBR-λN fusion in Xist-BoxB cells rescued the silencing defect. Likewise, a Lamin B1-λN fusion was able to synthetically tether ΔLBS-Xist-BoxB to the nuclear lamina and enable Xist to spread to active genes to a similar level as that observed in wild-type conditions.

To account for these findings Chen *et al*. (2016) proposed that Xist initially localizes at DNA sites in close 3D proximity to the Xist locus. The Xist-coated DNA binds to LBR when it comes into spatial proximity to the nuclear lamina and becomes tethered there. This moves the Xist-coated DNA away from the actively transcribed Xist locus, and enables other DNA regions on the X chromosome that are physically linked to these tethered regions to be brought into closer spatial proximity of the Xist transcription locus. In this way, Xist and its silencing factors can spread to these newly accessible DNA regions on the X chromosome.

Tsai *et al*. (2016) generated LBR knockout HeLa cells (LBR KO) using the CRISPR/Cas9 system. The LBR KO cells displayed normal cell and nuclear envelope morphology, normal growth, and normal localization of other nuclear envelope (NE) structural proteins, including Lamin B1, Lamin A/C, and Emerin. However, they were more sensitive to growth in cholesterol-limiting medium than the control WT HeLa cells, despite possessing an apparently wild-type TM7SF2 gene, which encodes the putative second sterol reductase. The LBR KO cells exhibited cell death within 5–7 days on cholesterol-limiting medium, and this effect was rescued by addition of 10 μM exogenous cholesterol to the medium. The cholesterol-auxotrophy phenotype was complemented by transfection with wild-type LBR, the C-terminal sterol reductase domain, or by a fusion protein that joined the nuclear domain of Sun2 (containing a nuclear targeting signal) to the LBR CTD. But it was not complemented by transfection with the LBR nucleoplasmic domain and only the first transmembrane helix of the CTD (LBR TM1), or by constructs bearing the disease-associated LBR point mutations N547D and R583Q. These point mutations were found to reduce sterol C14 reductase activity by decreasing LBR's affinity for the cofactor NADPH. Similar results were obtained in HeLa, HEK293T and human foreskin fibroblast (HFF) cells transfected with LBR siRNA but not by control siRNA. Thus, in at least three different human cell types LBR did not appear to play a significant role in NE organization, possibly because it is functionally redundant with other lamina-associated membrane proteins, yet despite the presence of TM7SF2 its sterol reductase activity was required for cell viability under sterol-restrictive growth conditions.

Two other LBR mutations, LBR 1402TΔ and LBR 1600*, encoded truncated proteins that were ubiquitylated and rapidly turned over by a novel nuclear membrane-based protein quality control pathway. It was previously not known that proteins can be degraded at the inner membrane of the nuclear envelope of mammalian cells. Thus, the LBR mutants may turn out to be useful tools to investigate how this happens. Other human diseases resulting from mutations in proteins of the nuclear envelope or lamina can now be examined from the perspective of protein quality control.

A provisional model proposed by Nikolakaki *et al*. (2017) posits that LBR's role in nuclear architecture might depend on both its sterol reductase activity to provide the necessary cholesterol or its derivatives to assemble lipid rafts in the INM, and on the capacity of LBR to form various multimers which are accommodated in these lipid microdomains and bind nuclear components essential to tether heterochromatin to the INM. They suggested it might be useful to probe whether the sterol reductase activity is affected by post-translational modifications within LBR's N-terminal nucleoplasmic domain (phosphorylation, O-GlcNAcylation, and others). It might also be worth examining whether the LBR KO can be complemented by a fusion of LBR NTD to chimeric CTDs made from segments derived from TM7SF2 and the LBR CTD.

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