An Introduction to Laforin and Malin

Learning about Laforin and Malin, the proteins that lose function in Lafora disease patients.

RESEARCH SIMPLIFIED BY

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QUESTIONS?

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Articles on Laforin:
https://pubmed.ncbi.nlm.nih.gov/36511140/
https://pubmed.ncbi.nlm.nih.gov/25544560/
Articles on Malin:
https://pubmed.ncbi.nlm.nih.gov/22815132/
https://pubmed.ncbi.nlm.nih.gov/31523006/

LAFORA DISEASE AND LAFORA BODIES

The brain is very sensitive to changes in sugar availability, controlling sugar levels to prevent cell death. Glycogen is an energy storage molecule that allows the brain to quickly access sugar when energy levels are low. Proteins play an important role in extracting sugars from glycogen. When proteins like laforin and malin lose their function, the sugar cannot be extracted, forming aggregates that are toxic energy molecules. These aggregates have long branches covered in phosphate molecules **(Figure 1)**.

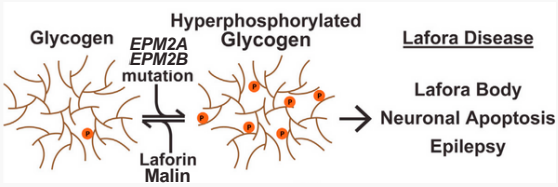

Figure 1. *Diagram of glycogen molecules in Lafora disease progression. Lines represent glycogen branches, ℗ represents phosphate groups attached to the glycogen molecule*
Image adapted from Dr. MK Brewer

Due to their long branches, these aggregates do not dissolve in water, forming aggregates as they clump together, and in fact, have a similar shape to amylopectin, a component that makes up starch in plants. In the brain, these aggregates are known as Lafora Bodies (**LB**), forming aggregates in the brain, a hallmark of Lafora disease (**LD**) which is a rare fatal progressive myoclonic epilepsy.

Over the years, there have been many challenges to studying the proteins involved with glycogen metabolism, with over 46% of glycogen being depleted within 1 minute after extraction. However, with novel research technologies and animal models, there has been a rapid expansion in LD understandings.

LAFORIN, MALIN, AND LAFORA DISEASE

Studies on Lafora disease development discovered that LD arises from mutations in EPM2A (**laforin**) or EPM2B (**malin**). Laforin and malin are proteins that regulate glycogen's shape. In general, it is recognized that laforin binds to glycogen, acting as a platform for other proteins to join it, while removing phosphate molecules from glycogen. Malin was revealed to be an E3-ubiquitin ligase, interacts with the laforin platform on glycogen, marking proteins to be broken down or transported to prevent glycogen accumulation.

Mutations to either one of these genes result in the formation of LBs linked to Lafora disease onset. Interestingly, mutations to either gene leads to the same disease progression. The data indicate proteins are involved in the same biological pathway.

Since Lafora disease is driven by LB accumulation, Lafora disease is considered a glycogen storage disease (GSD). Additionally, the data suggest that some proteins also aggregate in Lafora disease, and could be considered a member of the group of protein clearance diseases which already includes Parkinson's, Huntington's, and Alzheimer's Diseases.

LAFORIN AND ITS FUNCTIONS

Laforin is a phosphatase, removing phosphate groups from glycogen. This protein is the only identified human phosphatase to target glycogen. Researchers visualized laforin's structure **(Figure 2)** and found that laforin has two key domains, and in cells, each domain is paired, resulting in a dimer (CBM1-DSP1-DSP2-CBM2). To understand each domain's function, researchers studied mutations:

- **Dual Specificity Phosphatase (DSP):** This is where laforin actually removes phosphates from glycogen, primarily targeting the C2 and C3 sites **(Figure 3)**. Through the study of mutations, researchers demonstrated that a mutation at C266 reduced protein function by 69-95%, identifying it as the site of protein activity. In addition, this DSP is similar to two plant phosphatases that remove phosphate groups from starch.
- **Carbohydrate Binding Module (CBM):** This contains the binding site where laforin connects to glycogen. This protein prefers to bind to long linear sugar chains found in glycogen. Patient mutations that were studied showed reduced binding capabilities by 40%.

The CBM and DSP subunits were found to interact very closely together for increased stability. When mutating the interaction regions, they noticed mutations caused a 47-93% decrease in function.

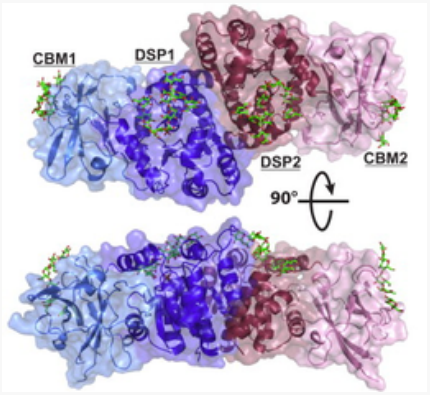

Figure 2. *Human laforin bound to maltohexose (green) with attached phosphate groups (orange). The protein has replicate halves, each with its own CBM and a DSP. (Raththagala, 2015)*

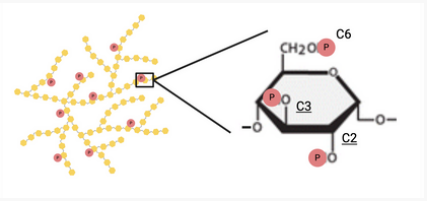

Figure 3. *Possible glycogen dephosphorylation sites, laforin's preferred locations are underlined*
Image created with BioRender

MALIN AND ITS FUNCTIONS

Malin is an E3 ubiquitin ligase, which specifically links the ubiquitin molecule to target proteins, marking them for degradation and transportation. Ubiquitination coordinates many processes in cells by using ubiquitin tags to signal protein destruction, moving cargo, and cell interactions **(Figure 4)**. Interestingly, when **RBCK1** (a similar E3 ligase) loses its function, it results in sugar aggregate accumulation resulting in a fatal skeletal and cardiac disease, revealing the critical role of E3's for maintaining levels of sugar storage and protein in the cell.

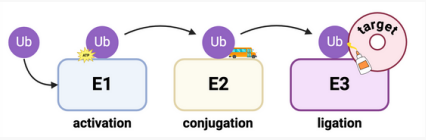

Figure 4. *The ubiquitination steps, essentially passing along ubiquitin (Ub) between proteins. Malin attaches Ub to target proteins*
Image created with BioRender

It was found that Malin has two domain in its structure **(Figure 5)**:

- **RING finger domain:** This is where malin does its work as an E3 ubiquitin ligase, attaching ubiquitin to target proteins and mark them for degradation or transportation to another part of the cell.
- **NHL-protein interaction domain:** These six repeated motifs are linked to form a propellor-like shape, capable of recognizing targeted proteins including laforin. This domain is very similar to TRIM32, another E3 ubiquitin ligase.

In the context of Lafora disease, *EPM2B-LD* patients most commonly have the malin-D146N mutation which interferes with malin's interactions with laforin; but not completely, resulting in a slower LD progression.

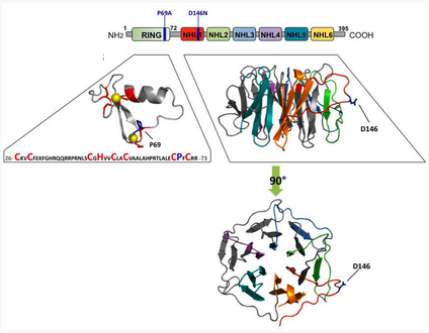

Figure 5. *Human malin protein depicting the RING (left) and NHL domains (right) which which has a β-propeller shape (below). The most common EPM2B-LD mutations are labeled (top)*
(Romá-Mateo, 2012)

LAFORIN AND MALIN INTERACT WITH EACH OTHER

Many studies have attempted to understand how malin comes together with laforin by studying its evolutionary history:

- **Laforin** has been around for a long time, longer than malin, having been found different types of species. It is capable of working independently on glycogen.
- **Malin** is a newer protein. Given the structural similarities, it is likely malin came from TRIM32, an older E3 ligase, and evolved to tag other proteins. However it requires other proteins to target glycogen and fulfill its function.

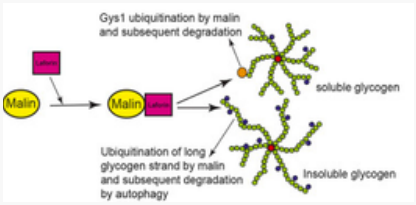

Figure 6. *Malin can interact with glycogen when it forms a complex with laforin (Mitra, 2023)*

Malin is found to interact with laforin, forming a laforin-malin complex to work as a team. In fact, malin relies on laforin in order to interact with polyclugosans, only then can malin do its work **(Figure 6)**. However more data is still needed to understand how this mechanism occurs

LAFORIN-MALIN COMPLEX CONTROLS GLYCOGEN METABOLISM

While it has many interactions with glycogen and aggregates, the Laforin-Malin complex has been found to work with a variety of other molecules. Another major molecule that the complex works with is R5/PTG, which is essentially a power switch involved in glycogen metabolism (build up and breakdown). Working as the 'on' switch, R5/PTG it plays the role forming of glycogen in cells by doing the following:

- It turns on **glycogen synthase 1 (GYS1)** which creates glycogen, effectively turning up the faucet **(Figure 7)**
- It shuts off **glycogen phosphorylase (PYGB)** which breaks down glycogen, effectively closing the drain **(Figure 7)**

Typically the Laforin-Malin complex **turns down R5/PTG**, limiting glycogen production. But when the Laforin-Malin complex does not work properly, R5/PTG stays on, accumulating LBs characteristic of Lafora disease.

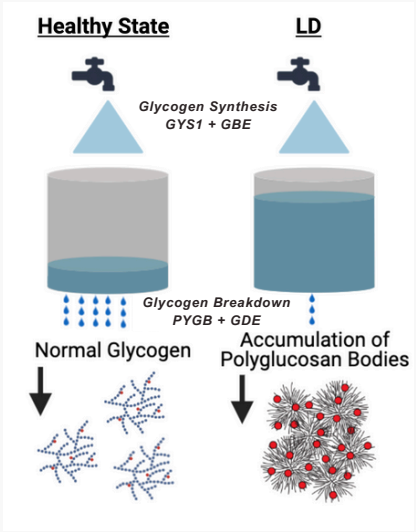

Figure 6. *Visualization of polyglucosan accumulation, GYS1 and GBE make glycogen while PYGB and GDE break down glycogen*
Image adapted from Dr. Matt Gentry

Overall, the Laforin-Malin complex regulates glycogen metabolism to **lower glycogen levels** by:

- Ubiquitinating R5/PTG, clearing out this 'on' switch to reduce its glycogen synthesis activity
- Ubiquitinating GYS1 and GDE, taking out out the proteins that create glycogen
- Ubiquitinating neuronatin, which removes yet another 'on' switch for creating glycogen

Since the Laforin-Malin complex is involved with regulating glycogen production process at various points, it is clear that if the complext is dysfunctional as a result of mutations to laforin-EPM2A or malin-EPM2B, that toxic, poorly branched aggregates will begin to accumulate, creating toxic brain environment that will result in Lafora disase development.

MALIN AND LAFORIN'S INVOLVEMENT IN BRAIN PROTECTION

In typical scenarios, laforin and malin have shown their abilities in protecting the brain. One of the ways these proteins play their role in protecting the brain is by working with heat shock protein 70 (**Hsp70**) which removes faulty proteins. Hsp70 works with malin to ubiquitinate these harmful proteins for their removal. However with the loss of laforin or malin function, these misfolded proteins begin to accumulate, leading to increased cell stress and cell death. The pathway affecting LD development is similar, with mutations to genes encoding laforin or malin leading to the accumulation of harmful glycogen-based Lafora Bodies.

Laforin and malin are also known to help activate **autophagic processes**, which clears out harmful cells. However, when laforin or malin lose their function, autophagy becomes dysfunctional which stresses brain cells. Autophagy dysfunction has been observed as one of the earliest signs in Lafora disease models, with malin-deficient mice showing autophagy dysfunction as early as 16 days old.

This impaired relationship with autophagy is a significant finding, making Lafora disease increasingly similar to other more common disorders including Parkinson's, Alzheimer's, and Huntington's disease. In fact there has been discussions to give Lafora disease a designation as a protein clearance disease, potentially joining the group of the previously mentioned disorders

LAFORIN-MALIN COMPLEX HAS INTERESTING INTERACTIONS

When assessing the physical structures of laforin and malin when they come together to form a complex, researchers determined that both of these proteins contained regions designed to allow them to work together as a unit in the Laforin-Malin complex. Researchers found that malin-D146N, a common LD mutation, completely disconnected laforin from malin, preventing them from working together. In addition, it was also found that in cases requiring glycogen breakdown (glucose starvation), AMPK (a signaling enzyme) activates laforin's S25 region, which increases laforin-malin interactions, but more data is needed.

It was also found that malin can ubiquitinate laforin to have it destroyed. When malin was mutated or deleted, laforin numbers had actually increased. There are currently two possible explanations for this:

1. Malin degrades laforin to have it replaced over time with new laforin
2. Laforin uses its CBM to hide in LBs, preventing it from being degraded

Regardless it is safe to say that malin is more stable with laforin, and more research is required

WHY THE LAFORIN-MALIN COMPLEX EXISTS

Researchers proposed two key hypotheses to explain the origins of the Laforin-Malin complex. Although these ideas vary in their approaches, the have the same final result: clearing out LB aggregates

Hypothesis #1, The Laforin-Malin complex is designed to protect the brain against aggregates:
Laforin scans GYS1 and GDE levels, and creates a scaffold for malin to clear out GYS1 glycogen metabolism proteins when levels are too high

Hypothesis #2, Laforin finds aggregates to trigger an immune pathway to bring in malin:
Laforin finds aggregates before they become toxic to the brain and recruits malin, which ubiquitinates proteins to activate the autophagy process to clear out these aggregates.