

Review Article

IMPDH dysregulation in disease: a mini review

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Inosine-5'-monophosphate dehydrogenase (IMPDH) is a highly conserved enzyme in purine metabolism that is tightly regulated on multiple levels. IMPDH has a critical role in purine biosynthesis, where it regulates flux at the branch point between adenine and guanine nucleotide synthesis, but it also has a role in transcription regulation and other moonlighting functions have been described. Vertebrates have two isoforms, IMPDH1 and IMPDH2, and point mutations in each are linked to human disease. Mutations in IMPDH2 in humans are associated with neurodevelopmental disease, but the effects of mutations at the enzyme level have not yet been characterized. Mutations in IMPDH1 lead to retinal degeneration in humans, and recent studies have characterized how they cause functional defects in regulation. IMPDH1 is expressed as two unique splice variants in the retina, a tissue with very high and specific demands for purine nucleotides. Recent studies have revealed functional differences among splice variants, demonstrating that retinal variants up-regulate guanine nucleotide synthesis by reducing sensitivity to feedback inhibition by downstream products. A better understanding of the role of IMPDH1 in the retina and the characterization of an animal disease model will be critical for determining the molecular mechanism of IMPDH1-associated blindness.

Introduction

Inosine-5'-monophosphate dehydrogenase (IMPDH) is an essential enzyme in purine biosynthesis where it catalyzes the first committed step in GTP synthesis. The IMPDH enzymatic reaction is well characterized [1]. In cells, IMPDH assembles into large filamentous ultrastructures, and recent *in vitro* work has defined the mechanism of IMPDH self-assembly into filaments, and shown that assembly plays a role in allosteric regulation [2–6]. In humans, there are two isoforms of IMPDH that share 84% sequence identity: IMPDH2 is up-regulated in proliferating cells while IMPDH1 plays a house-keeping role and is expressed in most tissues [7–9]. Missense mutations in both genes lead to disease in humans. Mutations in IMPDH1 result in autosomal dominant blindness in the form of retinitis pigmentosa (RP) or Leber congenital amaurosis (LCA) [10–14]. Mutations in IMPDH2 were only recently discovered, and are associated with severe juvenile neuropathies [15,16]. The tissue-specific nature of the disease for each isoform is curious and may shed light on the role of IMPDH1 in the retina and IMPDH2 in neuronal development. Here, we focus on IMPDH1 mutations that lead to retinal degeneration because the effects of these mutations have been more well studied than the IMPDH2 mutations.

RP is a common form of inherited blindness that affects 1 in 3000 to 1 in 4000 people worldwide [17,18]. RP is a group of related eye disorders that are caused by mutations in over 70 genes [19,20], leading to a wide variety of disease mechanisms. The first symptom in patients, loss of night vision, generally appears during adolescence or adulthood and is followed by narrowing of the field of vision. RP is characterized by degeneration and death of rod photoreceptor cells followed by the death of cone photoreceptors [17,21]. More severe forms of RP exist, including LCA which has a much earlier onset, often during infancy, more severe vision decline, and accounts for 10–20% of childhood blindness [22,23]. While gene therapies are an active focus of clinical trials [24–26] there remain very few treatment options.

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Over 70 genes have been linked to RP many of which play a role in the phototransduction cascade, ciliary structure and transport, or RNA splicing [27,28]. In contrast, mutation of IMPDH1 in RP is intriguing since it is among a smaller subset of RP-linked genes that are involved in metabolism and the only gene that is involved in nucleotide biosynthesis [11,27,29]. Twelve missense mutations in the coding region of IMPDH1 have been reported that are linked to RP, but until recently there was very little insight into the molecular mechanisms of disease for IMPDH1-linked RP [10–14].

IMPDH structure and function

IMPDH is an essential enzyme in GTP synthesis where it converts IMP to XMP (Figure 1A). The IMPDH monomer has two domains: the catalytic domain where IMP and NAD⁺ are converted to XMP and NADH and the regulatory Bateman domain [1,30]. The regulatory domain can bind adenine and guanine nucleotides at three distinct binding sites [2,4,31]. Site 1 has a preference for ADP/ATP, while site 2 can bind either ADP/ATP or GDP/GTP, and site 3 is exclusively GDP/GTP (Figure 1B). In solution, IMPDH is a constitutive tetramer, and nucleotide-binding drives reversible dimerization of the regulatory domains resulting in the assembly of an octamer (Figure 1C). If GTP is present, GTP binds in sites 2 and 3 where binding in site 3 promotes the octamer to compress into a lower activity state [2,5] (Figure 1D).

In vertebrate cells, IMPDH forms filamentous ultrastructures in response to the high demand for guanine nucleotides [32–34]. Activation of T-cells also drives the assembly of IMPDH into filaments [35,36]. Given that the isoforms have a high degree of sequence similarity and most antibodies cannot distinguish between isoforms, it is challenging to determine which isoform(s) are in these ultrastructures in cells in immunofluorescence experiments [32,37–43]. Immunogold labeling of fixed cells suggest that IMPDH ultrastructures are made up of shorter filaments that laterally interact to form bundles [44,45]. *In vitro*, both IMPDH isoforms assemble into single stranded filaments [3,4,46]; why these single stranded structures tend to aggregate into bundles in cells remains unclear, and may be the result of macromolecular crowding, or be driven by interactions with other cellular factors. Thorough characterization of IMPDH filaments *in vitro* has revealed that nucleotide binding to the regulatory domain promotes self-assembly of filaments made of stacked octamers (Figure 1E) [3,4,46]. IMPDH2 filaments serve a regulatory function, allowing the protein to resist GTP-feedback inhibition [5] while canonical IMPDH1 filaments have no effect on activity or inhibition [6].

The two IMPDH isoforms have different compressed filament architectures [5,6]. ATP binding in sites 1 and 2 in the regulatory domain, drives assembly of active IMPDH filaments in the extended octamer state that are identical for IMPDH1 and IMPDH2 (Figure 2A,B) [5,6]. GTP binding at regulatory domain sites 2 and 3 induces two conformation changes: compression of the octamer and flexing of the catalytic domain to an active ‘flat’ or partially inactive ‘bowed’ conformation. In IMPDH2, GTP-dependent filament assembly stabilizes the flat tetramer conformation, which allows IMPDH2 to remain partially active in the filament, even in a compressed GTP-bound state (Figure 2A) [5]. The GTP-bound IMPDH2 filament has the same assembly contacts as the ATP-bound IMPDH filaments, so that the only change between states is the compression within each octamer. IMPDH1, on the other hand, forms inhibited filaments with a completely different, and much smaller, assembly interface resulting in a new filament architecture that can only accommodate the inactive, ‘bowed’ tetramer conformation [6] (Figure 2B). Because the IMPDH1 bowed tetramer is found in the GTP-bound filament, polymerization of IMPDH1 has no effect on enzyme activity or sensitivity to feedback inhibition. Given that the isoforms have high sequence similarity and most filament contacts are preserved, presumably IMPDH1 and IMPDH2 could form mixed higher-order structures, but this has yet to be explored.

IMPDH’s canonical role is in purine biosynthesis but it may have other essential functions in the cell. IMPDH has been reported to associate with chromatin and telomeres [47,48], to bind single stranded nucleic acids with high affinity [49], to act as a transcription factor [50], associate with ribosomes [51], and IMPDH ultrastructures have been observed in nuclei [45]. These findings suggest that IMPDH may function in transcription regulation [52], but no clear model that integrates IMPDH’s enzymatic and potential transcriptional roles has emerged.

IMPDH in the retina

IMPDH1 is the major isoform in the retina. In mammals, retinal IMPDH1 is expressed as two major splice variants [53–55]. Both mammalian IMPDH1 retinal splice variants maintain the core canonical protein, but additional exons add residues to the C-terminus or both the C- and N-termini (Figure 3A). The retinal variants are named for the number of amino acids. In humans, the shorter retinal variant is IMPDH1(546) which has

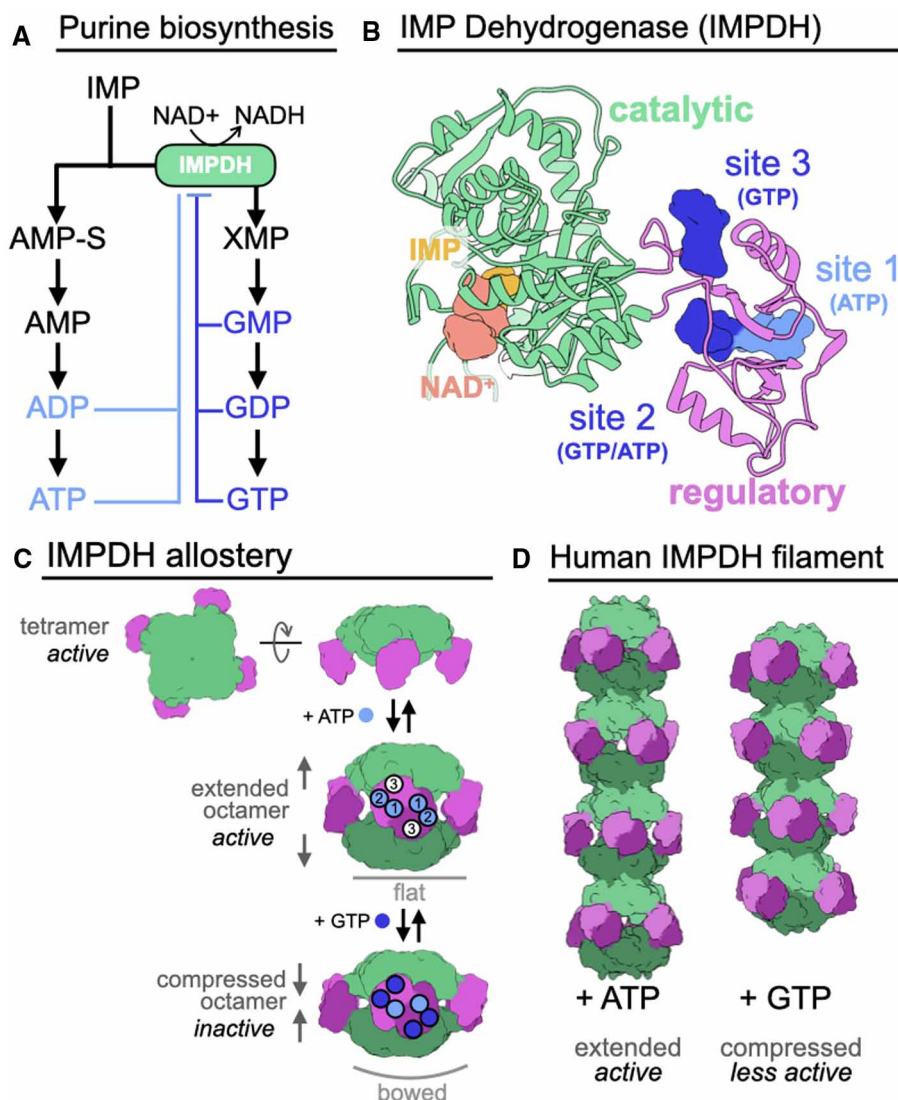


Figure 1. IMPDH structure and function.

(A) Purine biosynthesis pathway. (B) IMPDH monomer (6u9o) has a catalytic domain (green) that binds IMP (gold) and NAD⁺ (salmon) in the active site, and a regulatory domain (pink) with three allosteric nucleotide binding sites that bind GTP (dark blue) and ATP (light blue). The monomer is shown in the GTP-bound inhibited state. (C) In solution IMPDH is a tetramer, binding of ATP (sites 1 and 2) or GTP (sites 2 and 3) promotes octamer assembly. In the presence of ATP, octamers are extended and when GTP is bound in site 3, octamers are compressed (D) IMPDH octamers can assemble into filaments of stacked octamers. Figure adapted from [6].

five residues at the canonical C-terminus replaced by 37 new mostly unstructured residues [53]. IMPDH1(546) is the more common retinal variant in humans [53]. The other human retinal variant IMPDH1(595) has the same C-terminal extension, plus an additional 49 residues at the N-terminus. Mouse and human retinal variants have reduced sensitivity to GTP inhibition compared with the canonical variant [6,56,57]. The N- and C-terminal retinal splice variant extensions independently contribute to the reduced sensitivity to feedback inhibition by GTP [6] which is consistent with the high guanine nucleotide demand in photoreceptors [58].

We recently determined cryo-electron microscopy (cryo-EM) structures of IMPDH1 splice variants in multiple liganded states that shed light on the functional differences among them [6]. In the presence of ATP or GTP, both retinal variants assemble filaments of stacked octamers that are broadly similar to the canonical protein [6]. IMPDH1(546) extended/flat and compressed/bowed filaments are nearly identical with the

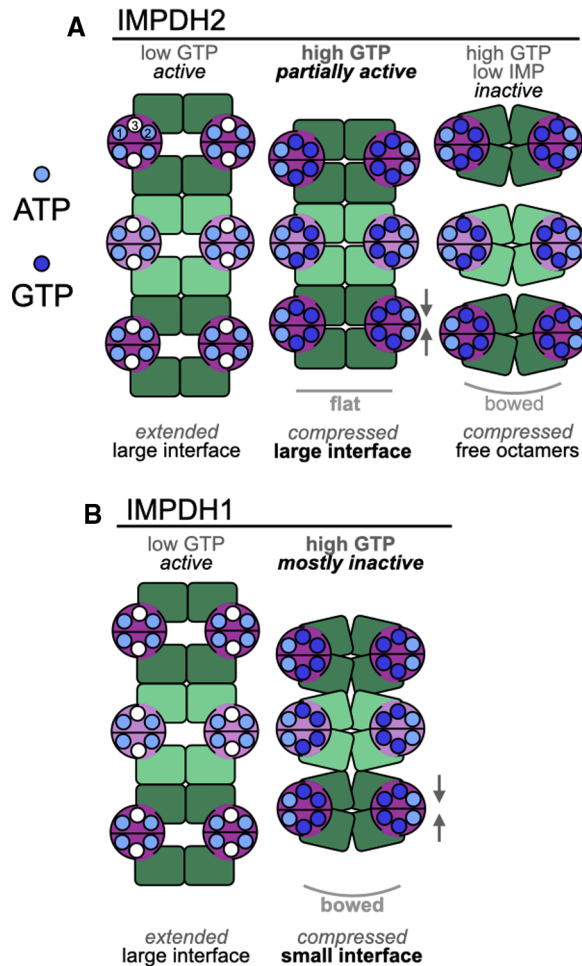


Figure 2. Model of IMPDH1 isoform assembly and filament role in regulation.

(A) In the presence of ATP, IMPDH2 assembles extended octamer filaments. Binding of GTP leads to assembly of compressed octamer filaments that remain partially active where the tetramer is in a flat conformation. In the presence of very high GTP concentrations, the tetramer is completely inhibited and enters a bowed conformation which promotes disassembly of the filament into free octamers [5] (B) For canonical IMPDH1, binding of ATP drives assembly of a filament composed of extended octamers. In the presence of GTP, canonical IMPDH1 assembles into a filament with the small interface made of fully compressed octamers that are mostly inhibited and have the bowed tetramer conformation. Figure adapted from [6].

canonical IMPDH1 protein and the C-terminal addition is not resolved in cryo-EM maps [6]. Despite no apparent structural difference from canonical IMPDH1, IMPDH1(546) has an increased IC_{50} for GTP. One likely explanation for reduced GTP-sensitivity is that the flexible C-terminal addition sterically hinders the octamer compression needed for complete inhibition.

IMPDH1(595) extended octamer filaments are very similar to canonical IMPDH1 and IMPDH1(546), with one striking difference — a short 10-residue helix in the N-terminal extension in IMPDH1(595) which sits at the filament assembly interface where it stabilizes inter-octamer contacts and the flat tetramer conformation (Figure 3B). Unlike canonical IMPDH1 and IMPDH1(546), which both when bound to GTP transition to the filament with the small assembly interface that has the compressed/bowed architecture, this additional helix locks IMPDH1(595) in the large interface, so that GTP binding promotes compression but retains the flat partially active tetramer conformation (Figure 3B). Consistent with a role in stabilizing the partially active filament conformation, the effect of the N-terminal extension on reducing sensitivity to GTP is completely dependent on the ability to form polymers [6]. The effect of the splice variants decreasing sensitivity to high GTP concentrations is congruent with high GTP concentrations in the retina [58–61].

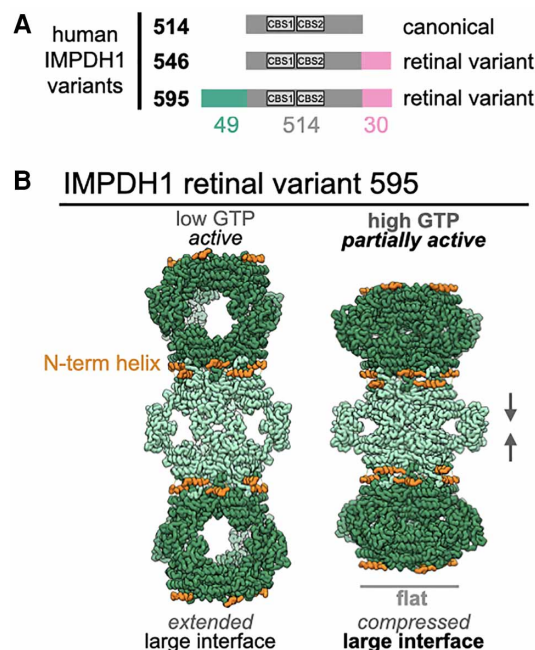


Figure 3. IMPDH1 retinal splice variants form filaments.

(A) Representation of IMPDH1 variant sequences. (B) Low-pass filtered cryo-EM reconstruction of IMPDH1(595) with octamers colored in green and the 10-residue helix found in the N-terminal addition in orange. For IMPDH1 retinal variant 595, the binding of ATP drives assembly of a filament with the large interface composed of extended octamers. GTP binding drives assembly of an IMPDH1(595) filament composed of the large interface, compressed octamers that are partially inhibited, and have the strained tetramer conformation. In both filaments, the N-terminal extension adds buried surface area to the large interface and the C-terminal extension is disordered.

Three phosphorylation sites were recently described in retinal IMPDH1 [61] (Figure 4A). One phosphorylation site, T159/S160, is preferentially phosphorylated upon light exposure (Figure 4B) but it is not clear which residue is phosphorylated. Therefore, the potential effect at each site must be considered. The phosphorylation site T159/S160 is in the regulatory domain where T159 points directly into nucleotide site 1 while S160 is near both site 1 and site 2, suggesting that phosphorylation at T159/S160 prevents nucleotide binding in site 1 and possibly site 2. Indeed, the phosphomimetic mutations T159D/E and S160D/E in canonical IMPDH1 resist GTP inhibition [61], suggesting phosphorylation at these sites disrupts GTP binding which may affect octamer conformation or filament assembly [31,46]. *In vivo*, increased phosphorylation at site T159/S160 is associated with increased supramolecular assembly [61]. Future studies to determine if phosphorylation at T159/S160 disrupts nucleotide binding and if it influences protein conformation would be informative. The second phosphorylation site S416 is on a loop near the active site whose movement is necessary for catalytic activity [1] (Figure 4C), and the phosphomimetic mutation S416D severely decreases V_{max} [61]. The third phosphorylation site S477 is preferentially phosphorylated in the dark. S477 is located at the filament assembly large interface (IMPDH1(546) extended filament and both IMPDH1(595) extended and compressed filaments) [6] and we predict phosphorylation here would disrupt filament assembly (Figure 4D). This site is particularly compelling because it is not involved in the interface of the GTP-bound IMPDH1(546) filament [6]. The consequences of these phosphorylations on filament assembly have yet to be characterized *in vitro*. Flux through the *de novo* purine biosynthesis pathway increases with light, and the observed light-dependent phosphorylation patterns [61] suggest a model for regulating IMPDH1 activity. In this context, phosphorylation of T159/S160 after a period of bright light exposure likely promotes retinal IMPDH1 to resist GTP inhibition and increase flux to guanine nucleotides while this flux is reduced in the dark by phosphorylation at S477 which we suspect contributes to the disassembly of IMPDH1 ultrastructures into more readily inhibited tetramers.

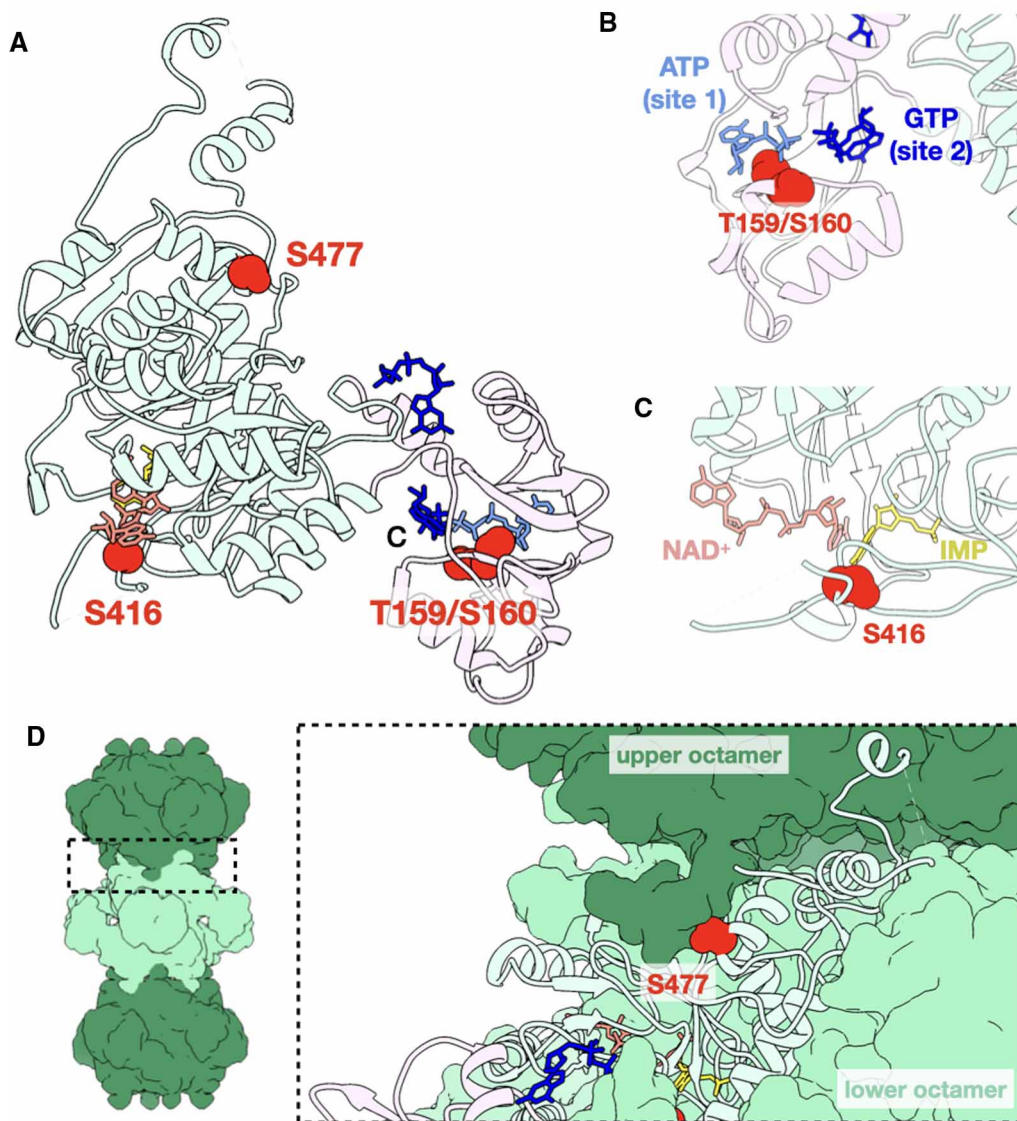


Figure 4. IMPDH1 retinal phosphorylation sites.

(A) IMPDH1 monomer (7rgd) with retinal phosphorylation sites [61] shown in red spheres. (B) Zoomed in view of phosphorylation site S416 showing its proximity to the active site with NAD⁺ in salmon and IMP in gold. (C) Phosphorylation site T159/S160 is between nucleotide binding sites 1 and 2 in the regulatory domain and might disrupt binding at either/both site(s). (D) Surface representation of IMPDH filament and zoom in of the interface between octamers where phosphorylation site S477 is nestled.

IMPDH1-associated retinitis pigmentosa

Twelve mutations in IMPDH1 lead to RP in humans, a subset of which lead to the more severe and early onset LCA [14] (Table 1, Figure 5A). Although IMPDH1 is expressed in most tissues, the only IMPDH1-related disease occurs in the retina. One explanation is that the retina requires a very specific balance of purine nucleotides including cyclic GMP and ATP [58]. Cyclic GMP is the key signaling molecule in the phototransduction cascade [62–64] while photoreceptors have an exceptionally high demand for ATP [65]. On top of this, there is a lack of redundancy in purine nucleotide production as there is very little expression of both IMPDH2 [53,66] and the major purine salvage enzyme HPRT [67,68]. Imbalanced purine pools in photoreceptors can lead to cell death [69,70]. Together, we predict this makes the retina exceptionally sensitive to any defects in IMPDH1's role in purine biosynthesis

Table 1. Characterization of IMPDH1-linked RP mutations

	GTP/GDP inhibition			<i>In vitro</i> assembly			<i>In vivo</i> expression	<i>In vitro</i> phosphorylation at 159/160	Single-stranded nucleic acid binding	Ribosome association			RNA binding in tissue culture
	Canonical	(546)	(595)	Canonical	(546)	(595)				Canonical	(546)	(595)	
R105W	N ^{1,2}	N ¹	N ¹	<i>Apo bundles</i> ³	N ³	N ³	–	N ⁶	<i>Decreased specificity</i> ⁷	–	–	–	–
T116M	N ¹	N ¹	N ¹	N ³	N ³	N ³	–	–	<i>Decreased specificity</i> ⁷	–	–	–	–
N198K	<i>Not inhibited</i> ^{1,2}	<i>Not inhibited</i> ¹	<i>Not inhibited</i> ¹	<i>Not compressed with GTP</i> ³	<i>Not compressed with GTP</i> ³	<i>Not compressed with GTP</i> ³	–	<i>Large reduction</i> ⁶	<i>Decreased specificity</i> ⁷	–	–	–	–
R224P	<i>Not inhibited</i> ^{1,2}	<i>Not inhibited</i> ¹	<i>Not inhibited</i> ¹	<i>No filament assembly</i> ³	<i>No filament assembly</i> ³	<i>No filament assembly</i> ³	<i>Guanosine-resistant bundles</i> ⁵	<i>Large reduction</i> ⁶	N specificity ⁸ <i>Decreased affinity</i> ⁸	–	–	–	<i>Decreased</i> ⁸
L227P	<i>Not inhibited</i> ²	–	–	–	–	–	–	–	–	–	–	–	–
D226N	<i>Not inhibited</i> ^{1,2}	<i>Not inhibited</i> ¹	<i>Not inhibited</i> ¹	<i>Apo bundles</i> ^{3,4}	–	–	<i>Guanosine-resistant bundles</i> ⁵	N ⁶	<i>Decreased specificity</i> ⁸ <i>Decreased affinity</i> ⁸	N ⁹	N ⁹	<i>Decreased</i> ⁹	<i>Decreased</i> ⁸
R231P	<i>Not inhibited</i> ^{1,2}	<i>Not inhibited</i> ¹	<i>Not inhibited</i> ¹	<i>Not compressed with GTP</i> ³	<i>Not compressed with GTP</i> ³	<i>Not compressed with GTP</i> ³	<i>Guanosine-resistant bundles</i> ⁵	–	–	–	–	–	–
K238E	<i>Not inhibited</i> ^{1,2}	<i>Not inhibited</i> ¹	<i>Not inhibited</i> ¹	<i>Not compressed or no filaments with GTP</i> ³	<i>Not compressed or no filaments with GTP</i> ³	<i>Not compressed or no filaments with GTP</i> ³	–	–	–	–	–	–	–
K238R	–	–	–	–	–	–	–	–	–	–	–	–	–
V268I	N ^{1,2}	N ¹	N ¹	N ³	N ³	N ³	–	N ⁶	<i>Decreased specificity</i> ⁸ <i>Decreased affinity</i> ⁸	–	–	–	<i>Decreased</i> ⁸
G324D	–	–	–	–	–	–	–	–	N ⁷	–	–	–	–
H372P	N ^{1,2}	N ¹	N ¹	N ³	N ³	N ³	–	<i>Increased</i> ⁶	<i>Decreased specificity</i> ⁷	–	–	–	–

Summary of the conditions IMPDH1 disease mutations have been tested. Dashed line indicates there are no published results, bold text indicates a finding that is indistinguishable from wildtype, N stands for 'normal', while italic text indicates a behavior different from wildtype; ¹Ref. [6];

²Ref. [4];

³Unpublished — Burrell and Kollman;

⁴Ref. [46];

⁵Ref. [72];

⁶Ref. [61];

⁷Ref. [14];

⁸Ref. [71];

⁹Ref. [51].

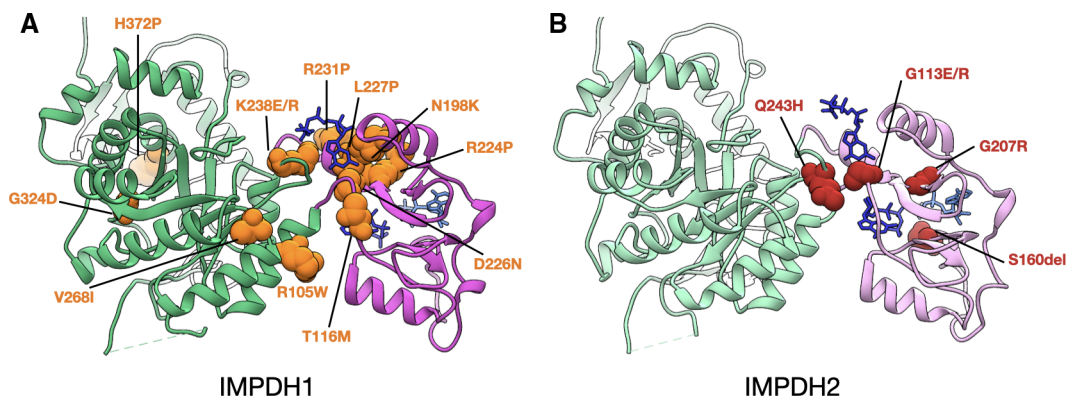


Figure 5. IMPDH disease mutations.

(A,B) IMPDH monomer with the catalytic domain in green and regulatory domain in pink. Regulatory domain is bound to two GTP molecules in dark blue and one ATP in light blue. (A) IMPDH1 (7rfg) with RP mutations in orange that cluster around the nucleotide binding sites. (B) IMPDH2 (6u9o) with neurodevelopmental mutations in red that cluster around the nucleotide binding sites.

Most of the IMPDH1 disease mutations map around nucleotide-binding sites 2 and 3 in the regulatory domain while the rest are scattered in the catalytic domain but distal from the active site (Figure 5A). A summary of all studies of the RP-associated mutations is in Table 1. Multiple studies have found that in the absence of ATP, the RP-associated mutations do not have an effect on specific activity [4,14,68,71]. Recent studies have focused on extending the initial results to study the mutations in all variants and found that the half-maximal concentration constant ($K_{0.5}$) to be similar between wildtype and all mutants [4,6] (Table 1). While 6 of the 10 disease mutations characterized were completely resistant to GTP-feedback inhibition (Table 1) [4,5]. The other four mutations (R105W, T116M, V268I, H372P) had no effect on feedback inhibition [4,6]. We recently showed that these effects were the same in canonical IMPDH1 and the two retinal splice variants [6]. There is no published functional data on the effect of the mutation K238R on the protein while mutation G324D has similar activity to the wildtype and does not affect binding to single stranded DNA [14]. These results have led to the hypothesis that at least 6 of the 12 mutations lead to photoreceptor degeneration due to the misregulation of purine biosynthesis [69,70].

The mechanism of disease of the other six RP-linked IMPDH1 mutations (R105W, T116M, V268I, H372P, K238R, G324D) remains unclear. One hypothesis is that these mutations disrupt a moonlighting role of IMPDH1. In the retina, these roles include binding single stranded DNA [14], associating with polyribosomes translating rhodopsin [51], or regulation by miRNA-34a [73]. R224P, D226N, V268I disrupt single stranded DNA binding *in vitro* and RNA binding in cell culture [69] but it is unclear what role IMPDH DNA/RNA binding plays in the cell and how disrupting this binding could lead specifically to photoreceptor cell death. One very compelling finding is that D226N in the retinal variant IMPDH1(595) disrupts association with polyribosomes [51]. Since IMPDH1 associates with ribosomes translating rhodopsin in the retina, this provides an exciting area for future work as mutations in rhodopsin are responsible for ~10% of autosomal dominant RP [74]. Understanding the structural details of the interaction between IMPDH and the mRNA or other protein that is facilitating the association with polyribosomes would allow for a more thorough understanding of how RP-linked mutations may or may not disrupt binding. A second hypothesis is that the mutations disrupt phosphorylation regulation. Plana-Bonamaisó et al. [61] demonstrated that the mutations N198K and R224P in IMPDH1(546) had significant reduction in phosphorylation at T159/S160 (Figure 4B) while H372P had increased phosphorylation at the same site. More research will need to be done to test the effect of all mutations on all three phosphorylation sites. These findings suggest that although via different molecular mechanisms, all IMPDH1 RP-associated mutations lead to disease through dysregulation of IMPDH1.

IMPDH2-associated neuropathy

A study from Zech et al. [15] first identified mutations in IMPDH2 associated with neurodevelopmental disorders. Similar to the GTP-resistant RP mutations in IMPDH1, these IMPDH2-disease mutations cluster around

nucleotide-binding sites 2 and 3 in the regulatory domain (Figure 5B). Therefore, it is reasonable to speculate that the IMPDH2-disease mutations might also disrupt GTP-feedback inhibition. Future studies will be necessary to characterize IMPDH2-disease mutants *in vitro* to test this hypothesis. It is intriguing that mutations in both isoforms lead to diseases in both the central nervous system and in highly specialized nerve cells in the retina, suggesting that there may be a unique dependence of neurons on finely balanced nucleotide biosynthesis.

Potential for IMPDH disease treatment

Treatment of IMPDH1-associated RP poses a unique challenge not only because it is autosomal dominant but also because there are only low levels of expression of the other isoform [53,66] in the retina to maintain purine biosynthesis. Therapeutic delivery to the retina is most commonly achieved by intravitreal injection. We can imagine two possible modes of therapeutic treatment. One option is to silence the mutant IMPDH1 allele. This can be done at the DNA level by disrupting the mutant gene or post transcriptionally through the use of antisense techniques such as RNAi [75]. Direct gene-editing techniques might prove useful as the retina is a fairly-accessible organ. The first clinical trial is currently underway using CRISPR technology to directly edit DNA in a small cohort of patients who have LCA10 [24,25]. A second treatment possibility for the class of mutations that resist GTP inhibition would be to treat the retina with pan IMPDH inhibitors that inhibit at binding sites distal from the mutation [76,77].

In a study by Tam et al. [78] they created the only animal model of IMPDH1-linked retinal degeneration by sub-retinally injecting an adeno-associated virus to mediate the expression of canonical IMPDH1 with the R224P mutation. Within 4 weeks of injection, they observed significant disruption of the photoreceptor layer [78]. Furthermore, the group successfully suppressed the negative pathological effects of R224P by disrupting IMPDH1 expression through the co-injection of a short hairpin RNA to IMPDH1. Additional animal models are needed to determine the molecular mechanism of disease and test other treatment options such as IMPDH inhibitors but this work demonstrates a strong possibility for successful treatment.

IMPDH2 neurodevelopmental disease treatment will depend on future *in vitro* characterization of the enzyme. If the disease-associated mutations disrupt feedback inhibition by GTP, then an inhibitor that binds distal from the GTP binding sites might prove an effective treatment. Given that most tissues express both IMPDH isoforms, it might be beneficial to use IMPDH2-selective inhibitors like sappanone [79] or shikonin [80].

Conclusion

The wealth of research on IMPDH has led to a detailed mechanistic understanding of allosteric regulation and structural rearrangements *in vitro*. Although the role of IMPDH1 retinal variants in the retina is beginning to be understood, there are still many unanswered questions. Robust *in vitro* characterization of IMPDH1-associated RP mutants has led to the hypothesis that 7 of 12 of the mutations disrupt the balance of purine nucleotides in the retina because they do not experience GTP-feedback inhibition. However, it remains unclear the molecular mechanism of disease for the remaining five point mutations but a few mutations have been shown to change phosphorylation and disrupt association with polyribosomes. More studies will be necessary to extend *in vitro* findings to the retina and an animal model will need to be pursued to develop a detailed understanding of the molecular mechanism of IMPDH1 dysregulation that leads to blindness.

Perspective

- Mutations in the ubiquitous enzyme IMPDH1 that self-assembles into filaments lead to autosomal dominant blindness in humans.
- IMPDH1 dysregulation results in retina degeneration; mutant-driven dysregulation occurs through different mechanisms including disruption of feedback inhibition, change in phosphorylation patterns, disruption of association with ribosomes, and others still to be identified.
- Characterization of an animal model with RP mutations in IMPDH1 retinal splice variants will be critical to understanding the molecular mechanisms of disease.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contributions

A.L.B. and J.M.K. wrote the manuscript.

Abbreviations

cryo-EM, cryo-electron microscopy; IMPDH, IMP dehydrogenase; LCA, Leber congenital amaurosis; RP, retinitis pigmentosa.

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