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<td>Authors</td>
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Figure 1. The *fenrir* mouse has brown fur (top). An unaffected littermate is shown (bottom).

*Fenrir* was initially identified among N-ethyl-N-nitrosourea (ENU)-induced G3 animals as a hypopigmentation mutant with brown fur (*Figure 1*).
Whole genome HiSeq sequencing of the G1 grandsire identified 61 mutations. Five G3 mice with the *fenrir* phenotype (and 23 unaffected mice) were genotyped at all 61 mutation sites and four mutations on chromosome 7 were homozygous in all five of the *fenrir* mice. The mutation in *Rab38* (LOD = 3.29) was presumed to be causative because the *fenrir* hypopigmentation phenotype mimics other known alleles of *Rab38* (see MGI for a list of *Rab38* alleles). Capillary sequencing of the mutated genes identified a T to A transversion at base pair 88450541 (v38) on Chromosome 7 in the GenBank genomic region NC_000073 encoding *Rab38*. The mutation corresponds to residue 263 in the mRNA sequence within exon 2 of 3 total exons.

247 GCTATGGGGGCATTTATGTTTTTGATGTCACC
83 -A--M--G--A--F--I--V--F--D--V--T--

The mutated nucleotide is indicated in red. The mutation results in a conversion of Isoleucine (I) to Asparagine (N) at position 88 (I88N) in the Rab38 protein.

**Protein Prediction**

![Flash Content]

**Figure 2. Domain structure of Rab38.** The six consensus GTP-binding sequences and the isoprenylation motif (CAKS) are shown at the top. Three of the GTP-binding sequences are involved in guanine (G1-G3) binding; the other three sequences are involved in phosphate/Mg\(^{2+}\) (PM1-PM3) binding. The positions of the RabF motifs (green) and RabSF motifs (pink) for Rab38 indicated; the gray bar indicates overlap. The *fenrir* mutation (shown by the red asterisk) is an isoleucine to an asparagine conversion at amino acid 88.
Rab38 encodes the highly conserved [human/rat (96.2%), human/mouse (93.8%), rat/mouse (95.2%)] 24 kDa Rab guanosine triphosphatase (GTPase). Rab38 [Figure 2; (1-3); reviewed in (4)]. The Rab proteins comprise the largest family within the Ras superfamily of small GTPases [see the record for concrete for information about Rab27a; reviewed in (4)]. Like all Ras superfamily proteins, Rab38 contains the six consensus sequences that mediate GTP-binding of Rabs [reviewed in (4:5)].

Three sequences are involved in guanine (G1-G3) binding [G1 = F; G2 = nXDX; G3 = EXSA; X is any amino acid], the other three sequences are involved in phosphate/Mg$^{2+}$ (PM1-PM3) binding [PM1 = GXXXXGGKs; PM2 = T; PM3 = DXXG] (6) [see Figure 2 for the locations of the PM/G motifs in Rab38]. The presence of five motifs designated as Rab family motif (RabF) 1-5 distinguishes the Rab proteins from other small GTPases (5). The RabF motifs are thought to mediate the interaction of the Rab proteins with Rab-specific regulators (e.g., Rab escort proteins (REP) and GDP dissociation inhibitors (RabGDI) [reviewed in (4)]. The RabF motifs cluster in and around the switch I and II regions [reviewed in (4)]. Small GTPases have switch I and switch II regions that change conformation as a GTPase switches between the GTP- and GDP-bound states [reviewed in (4)]. The prototypical amino acid sequences of the RabF motifs (and their corresponding location in the folded protein) are as follows: RabF1, IGVDF (loop 2 - ? 2, in the switch I region); RabF2, KLQI/LW (? 3); RabF3, RFRSIT (loop 4); RabF4, YYRGA (? 2-loop 5); RabF5, LYY/FDIT (? 4-loop 6) (5). RabF2-F5 cluster in and around switch II (between sheets ? 3 and ? 4) (5).

Four regions designated Rab subfamily motif (RabSF) 1-4 share high amino acid sequence identity among Rabs of the same subfamily. The RabSF regions are proposed to determine binding specificity to effectors, and to thus reflect functional similarity among members of a subfamily (7).

Prenylation is essential for Rab function in that it facilitates the localization of Rab proteins to specific cellular membranes. Most Rab proteins contain a C-terminal di-cysteine motif (e.g., XXXCC, XXCCX, XCCXX, CCXXX or XCCXC; C is cysteine and X is any amino acid) where both cysteines are modified by prenylation (i.e., the covalent addition of either geranylgeranyl (20-carbon) or farnesyl (15-carbon) isoprenoids via thioether linkages) (8;9). Other Rab proteins (e.g., Rab13, Rab23, Rab18, and Rab38) have a CAAX motif (A is an aliphatic residue) that is modified by a single geranylgeranyl moiety (10). Proteins with a C-terminal CAAX motif are prenylated by CAAX prenyltransferases (either farnesyltransferase or geranylgeranyltransferase type I) (11). When X is a methionine, serine, glutamine, or alanine, the substrate is farnesylated, whereas if it is a leucine or phenylalanine, the substrate is geranylgeranylated (11). The Rab geranylgeranyltransferase (RGGT, also known as geranylgeranyltransferase type II) specifically modifies Rab proteins that are in complex with the accessory protein Rab escort protein (REP), which also delivers prenylated Rabs to their target membrane (12).

Rabs containing only a single C-terminal cysteine are sometimes carboxyl methylated, thereby increasing the hydrophobicity of the protein. The same pathway is utilized by CAAX-containing Rab, Ras and Rho GTPases. After prenylation, RGGT dissociates and the AAX peptide is proteolytically cleaved by CAAX prenyl protease 2 (RCE1). The exposed prenylated cysteine is subsequently carboxyl methylated by ICMT (isoprenylcysteine carboxyl methyltransferase) at the ER membrane (11). For Rab proteins, carboxymethylation promotes the correct membrane association of the protein. In contrast, carboxymethylation of Rab proteins appears to affect only the distribution of the protein between membrane and cytosol. Although Rab38 was methylated in vitro, Rab38 was not carboxyl methylated in cells, indicating that Rab38 may not be a good RCE1 substrate in vivo (11). Subsequent site-directed mutagenesis to introduce a valine or leucine at the A2 position (lysine in CAXS) resulted in Rab38 methylation in vivo, indicating that the A2 position is an important determinant of RCE1-mediated proteolysis (11).

Cite this information as follows: Tiana Purrington, Anne Murray, Beutler B. Record for fenrir, updated Apr 25, 2018. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu

4 of 12
(Generated on Oct 23, 2018)
Although the crystal structure of Rab38 has not been solved, the Rab proteins share similar overall folds and Rab3A has been used as a model for the Rab proteins (13). The active form of rat Rab3A (amino acids 18-186) bound to nonhydrolyzable GTP analog GppNHp has been solved to 2.0 Å [Figure 3; PDB: 3RAB; (13)]. The 19-residue N-terminus is sensitive to proteolysis and was not included in the crystal structure (13). Rab3A consists of a six-stranded ?-sheet comprised of five parallel strands and one antiparallel strand surrounded by five ?-helices (13). The active conformation of Rab3A is stabilized by hydrophobic contacts between the switch I and II regions (13). Highly conserved serine residues in the phosphate-binding loop (P loop) and switch I interact with the ?-phosphate of GTP (13). Furthermore, these serine residues help stabilize the active conformation of the switch regions (13). The switch I?2 region (51FVSTVGIDFKV61) is highly conserved in exocytic Rabs (13). Thr54 (the underlined residue, above) mediates the interaction with the ?-phosphate (13). Several residues C-terminal to Thr54 are conserved among the Rab proteins and are analogous to the ‘effector-binding loop’ in Ras (54T(I/V)G(I/V/A)(D/E)F59) (13). The ?3-switch II region contains the highly conserved sequence 76WDTAGGER(Y/F)RTIT(S/T)(A/S)YYRGA95 and contains the DxxG motif common to all GTP binding proteins (13). The active conformation of Rab3A is mediated by interactions between the ?-phosphate of GTP and Thr54 in switch I and Gly80 in switch II (14).

The fenrir mutation results in the conversion of isoleucine 88 to an asparagine. Any changes in Rab38 expression and/or activity due to the mutation are unknown.

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5 of 12
(Generated on Oct 23, 2018)
Rab38 is expressed in mouse melanocytes, retinal pigment epithelial cells, alveolar pneumocytes, and platelets (2;15;16). Quantitative PCR of rat organs determined that the highest expression of Rab38 in the rat is in the lung, followed by skin, stomach, kidney, and liver (16). Whole-mount in situ analysis showed that Rab38 is expressed as early as embryonic day (E) 11.5 in melanocytes of the retinal pigment epithelium (RPE) (1).

Western blot for the rat Rab38 protein confirmed the mRNA expression pattern within lysosome-related organelle (LRO)-producing cells (i.e., melanocytes, platelets, and lung alveolar type (AT) II epithelial cells) (15-18). LROs share features with lysosomes, contain some lysosomal proteins, and have a low luminal pH. In the Brown Norway (BN) rat, Rab38 expression was detected in the kidney, heart and liver in addition to melanocytes, platelets, ATII cells and lung Clara cells (2). In mouse tissues, Rab38 was expressed in bone marrow mast cells and basophil-derived rat basophil leukemia (RBL) cells and at lower levels in lung and ATII–derived MLE-12 cells (18). Rab38 was not detected in mouse liver, brain, spleen, kidney, bone marrow dendritic cells, RAW (macrophage), AR42J (exocrine pancreas), MIN6 and INS-1 (insulinoma), or in AtT20 (anterior pituitary) cells (18). Observed differences in Rab38 localization in the liver and kidney between the rat and mouse have not been addressed.

Immunostaining of rat liver revealed that hepatocytes were stained for Rab38, while Glisson capsules were not (16). Within rat kidney, proximal renal tubular epithelial cells, but not the glomerulus were immunoreactive for Rab38 (16). Immunostaining for Rab38 in ATII cells revealed that Rab38 was distributed throughout the cytoplasm and colocalized with the ER resident protein BiP/GRP78; Rab38 did not colocalize with the Golgi and trans-Golgi network (17). Cell fractionation and imaging of EGFP-tagged Rab38 in isolated ATII cells showed that Rab38 targets to the limiting membranes of a subpopulation of lamellar bodies (19). Immunofluorescence staining of Rab38 in a differentiated megakaryocytic (i.e., bone marrow cells) line (Dami cells) detected perinuclear localization as well as punctate cytoplasmic staining attributed to granules (2). Rab38 partially colocalizes with Rab32, AP-3 complex, and clathrin in primary megakaryocytes and a human megakaryocytic cell line (MEG-01) (20); for information about the relationship between Rab38 and these proteins, see “Background”, below. Further analysis determined that Rab38 partially colocalized with Lamp-3, a protein localized to dense granules and lysosomes in resting platelets, and Lamp-1, a LRO marker (2).

In melanocytes, Rab38 was localized at the cell periphery (i.e., in mature pigmented melanosomes) and vesicular structures in the perinuclear region (1;18). Confocal immunofluorescence revealed that Rab38 (and Rab32) colocalize with AP-1, AP-3, and BLOC-2 complexes, in MNT-1 melanocytes (21;22). Rab38 and Rab32 associate with AP-3, AP-1, and BLOC-2 on the membranes, not in the cytosol, indicating that the GTP-bound form of the Rabs function in the assembly of the melanosomal complexes (21). Rab38 and Rab32 are proposed to localize to specific tubular domains of early/recycling endosomes that contain AP-1, AP-3, or BLOC-2 (20;21;23).

**Background**

For an overview of Rab GTPases please see the record for concrete.
Figure 4. Rab38 and the trafficking of proteins to lysosomal-related organelles (LROs) from the trans-Golgi network (TGN). BLOC-1 and AP-3 mediate early steps of vesicle trafficking from the early endosome, while BLOC-2 and BLOC-3 are involved in later stages. For simplicity, only the major cargo proteins affected by each HPS complex are shown (ATP7A, tyrosinase, Tyrp1, LAMP1). ATP7a in maturing melanosomes allows the influx of copper and activates tyrosinase. BLOC-1 also binds to the vesicle fusion protein syntaxin 13, which is localized to the stage I melanosome/coated endosome. BLOC-2 and AP-3 interact with clathrin. Melanosomal maturation is shown separately. During melanosome maturation, Rab32, Rab38, and Tyrp1 are necessary for the processing and trafficking of tyrosinase, a protein necessary for pigment production (inset).

Rab38 function in melanosomes
and 32 are highly homologous [Rab32 is 67% identical to Rab38 (18)] and have somewhat redundant functions in melanocytes (and other cells where LROs are present) [(15;18;21;24); reviewed in (25;26)]. Rab38 functions in the delivery of enzymes to the maturing melanosome in both skin melanocytes and in RPE cells (24). Rab38 and Rab32 cooperate with BLOC-2, AP-1 and AP-3 to assist tyrosinase [Tyr; see the records for ghost, pale rider, and siamese] and Tyr-related protein 1 [Tyrp1; (see the record for chi)]-containing vesicles from the trans-Golgi network to end-stage melanosomes in skin melanocytes and RPE cells to produce pheomelansins (yellow or red) and eumelanin (brown or black) pigment [(1;18;21;22;27;28); reviewed in (29)]. Deficiency in both Rab38 and Rab32 result in enhanced Tyrp1 recycling through the plasma membrane; Rab38 depletion does not lead to loss of Tyrp2 (Dct) expression, but Rab32 deficiency does (21). Rab38 also interacts with Varp, a guanine nucleotide exchange factor for Rab21, to facilitate the trafficking of Tyrp1 in melanocytes [(28;30); reviewed in (26)].

**Rab38 functions in kidney, lung, and platelet dense granules**

Rab38 functions in tubular reuptake and processing of filtered proteins in the proximal tubule of the rat kidney (3). The Fawn-Hooded Hypertensive (FHH) rat model carries a $\text{Rab38}$ mutation that results in a premature stop codon at codon one; the next in-frame ATG is in the second exon (2;15;31;32). Protein expression analysis by Western blot revealed no $\text{Rab38}$ protein expression in FHH tissues (2). The FHH model exhibits hypertension, proteinuria, and albuminuria and subsequently develop end-stage renal disease (3;33). Rescuing $\text{Rab38}$ expression in a Brown Norway (BN) strain expressing mutant $\text{Rab38}$ resulted in significant improvement in both proteinuria and albuminuria compared to levels observed in the FHH model (3;33).

Mutations in $\text{Rab38}$ lead to disruption in surfactant homeostasis in lung ATII cells as well as increased susceptibility to lung pathologies (e.g., interstitial pneumonia) and altered lung alveolar structures in both mice and rats (34;35). Rescue experiments in the FHH model revealed that Rab38 functions in regulating lamellar body morphology in ATII cells (19). Lamellar bodies, LROs in ATII cells, contain (and subsequently release once fused to cell membranes) surfactant proteins that function to lower alveolar surface tension, maintaining alveolar lumen in an open and relatively dry state (34).

Platelet dense granules (DGs) are storage organelles in platelets and contain ATP, ADP, serotonin, pyrophosphate, calcium, membrane-bound P-selectin (CD62P) and lysosomal-associated membrane protein (Lamp)-3 (CD63) (2). DGs are essential in platelet aggregation, adhesion and secretion in response to vascular injury (2). siRNA-mediated knockdown of $\text{Rab38}$ in the MEG-01 human megakaryocytic cell line led to deficient tethering or fusion (or both) of early endosomal cargo-containing vesicles with the immature DG (20).

**RAB38 is considered to be a Hermansky-Pudlak Syndrome (HPS)-related locus**

Defects in the trafficking of LROs due to mutations in genes associated with LRO biogenesis [e.g., $\text{HPS1}$, $\text{AP3}$ (see the record for bullet gray), $\text{HPS6}$ (see the record for stamper-coat), $\text{HPS3}$ (see the records for pam gray), $\text{HPS5}$ (see the record for dorian gray), $\text{CHS1}$ (see the record for souris), $\text{MYO5A}$ (see the record for new gray), and $\text{RAB27A}$ (see the record for concrete)] result in HPS-I-III, conditions in which patients can exhibit oculocutaneous albinism, bleeding diathesis, and progressive interstitial pneumonia (20;35;36). Although $\text{RAB38}$ mutations in human HPS patients have not been identified, it is considered to be an HPS-related locus (36-38).

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8 of 12
(Generated on Oct 23, 2018)
Several Rab38 mutant rodent models exhibit defective LRO biogenesis and are considered phenotypic models of HPS (1;15;18;32;35;36). The spontaneous mouse mutation chocolate (cht; Gly19Val; MGI: 1856819) is within the GTP-binding domain of Rab38 (1;2;15). Although the mutant Rab38 protein can still bind GTP, it is not prenylated and results in an inactive, unstable, and exclusively cytosolic protein (1;15;18;24;39). Homozygous cht mice exhibit oculocutaneous albinism characterized by a brown coat color, iris and RPE thinning, as well as altered homeostasis of lung surfactant, and aberrant ATII cell morphology; the cht mice do not exhibit platelet storage deficiency (15;24;34;36). The cht mice develop emphysematous lung disease (34;39). Quantification of Tyr in immature and mature melanosomes of the RPE determined that Tyr levels were reduced, indicating that Tyr delivery was defective in the cht eye (24). In addition, end-stage melanosomes from cht melanocytes expressed less Tyrp1 than control melanocytes, indicating that the cht mutation also impairs Tyrp1 sorting (1;36).

Several Rab38 mutant rat models have also been identified including ruby (R), FHH, and Cinnamon all of which carry an identical Rab38 mutation that results in a premature stop codon at codon one (2;15;31;32). Similar to cht, these models exhibit oculocutaneous albinism (“fawn” coat color in the FHH model), abnormal lung surfactant homeostasis, and aberrant lamellar body morphology (2;15;35;40). In contrast to cht, the rat models exhibit platelet storage deficiency and a lack of dense granules in megakaryocytes making them closer models of human HPS than the mouse mutants (35).

Similar to the other Rab38 mutant rodent models, fenrir exhibits albinism characterized by a brown coat color; eye color change in fenrir has not been observed. Although Rab38, Tyr, and Tyrp1 protein expression and localization have not been examined in fenrir, defects in the transport of Tyr and/or Tyrp1 are suspected. Other Rab38-associated phenotypes have not been studied and/or observed in fenrir.
Fenrir genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transition.

**PCR Primers**
Fenrir(F): 5’-CGGCAAGAGCCTACAACACGTGGAAC-3’
Fenrir(R): 5’-TTGCAGAAGCGGTCCATCTTGAGTC-3’

**Sequencing Primer**
Fenrir_seq(R): 5’-GAGTCCATTGTTGATAACACAGC-3’

**PCR program**
1) 94°C 2:00
2) 94°C 0:30
3) 55°C 0:30
4) 72°C 1:00
5) repeat steps (2-4) 40X
6) 72°C 10:00
7) 4°C ?

The following sequence of 505 nucleotides (from Genbank genomic region NC_000073 for linear DNA sequence of Rab38) is amplified:

19945       cggcaa gagcctacaa ctgttqaact atcttcaagg
19981 cccaataatc aactacctat tttttaaaaa ttaaattttc atttctgtgt ttaaatattta
20041 aaccaacatac ctacataaat gc ctatatttta tttctgtctct aatatattagc attaaacttt
20101 tcacattgtc acacctgtca tttggggggg atatgaagct ccagtgtagt gtacaattat
20161 gattctaatt ttgatgaatc ttttctgtctt gctgacaggt caagaaagat
20221 ttagtacttac ccgagagatc gacacattt ggcctggcgg ccagtttga ttttttggatg
20281 cccctacgag cgcctgatgt gggggggg ggtttagctgtt ggccaacattg atgtagtctt
20341 taacgctccc taatgtgtaag ccaatctgtg cctgctgtgt gctgctgctt ggtgacaggt cagggacagat
20401 ggaaggtgtctt gctttgaagct aatggaactca aatggaactca gttctgcaag

Primer binding sites are underlined and sequencing primer is highlighted; the mutated T is shown in red text.

**References**
Mutagenetix Phenotypic Mutation 'fenrir'


Retinal Pigment Epithelium Results in Instability of Immature Melanosomes during Transient Melanogenesis. *Mol Biol Cell.* 18, 3914-3927.


