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The Nramp2 G185R mutant iron transporter: defective in many ways

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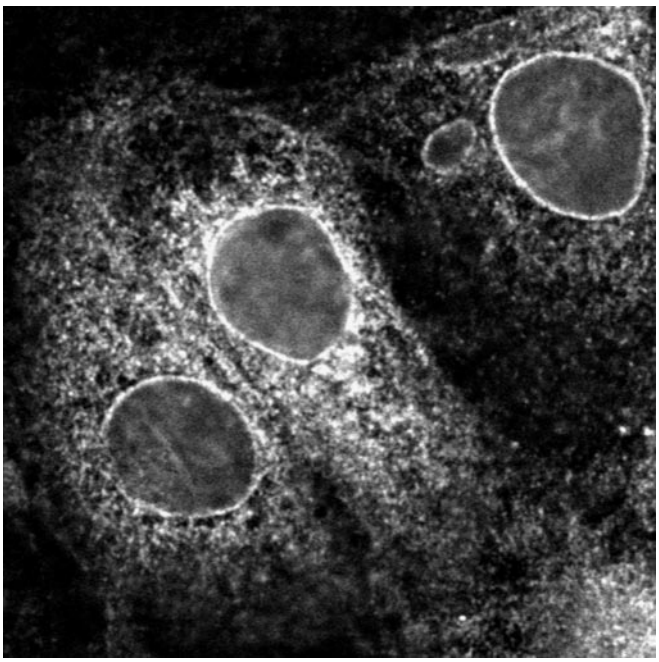
Abnormal processing, mislocalization, decreased stability, and diminished iron transport activity contribute to the functional deficiency and consequent iron deficiency seen with the G185R mutation in the metal transporter Nramp2.

Like other trace metal ions, iron is incapable of crossing cellular membranes by simple diffusion. The only identified transporter capable of mediating cellular iron uptake in higher eukaryotes is the integral membrane protein Nramp2 (also called DMT1 and DCT1). While Nramp2 transports certain other divalent metal ions as well, its role in iron uptake is most salient. Mutation of Nramp2 is responsible for the severe iron deficiency anemia recognized in 2 rodent populations—the *mk* mouse and the *Belgrade* rat. Curiously, the same glycine-to-arginine (G→R) amino acid substitution in Nramp2 is found in both mouse and rat models. Moreover, this G185R mutation has arisen independently in 2 different inbred mouse strains. The multiple independent occurrences of this mutation suggest the possibility of a gain-of-function and selective advantage for the mutant Nramp2 protein. Indeed, recent evidence demonstrates that Nramp2 G185R, unlike wild-type Nramp2, has the ability to transport calcium.[1] No other Nramp2 mutations have been reported, and it appears that a complete knockout of Nramp2 in mice results in a newborn lethal phenotype (communication cited in Xu et al[1]). These observations suggest that G185R Nramp2, although defective, may have residual iron transport properties. Thus, there is considerable interest in the cell biology and functional properties of G185R Nramp2 mutant protein.

Previous investigations have identified 2 different mechanisms by which the G185R mutation may be deficient as an iron transporter. Su et al found that Nramp2 G185R was functionally inactive as an iron transporter when heterologously expressed in HEK293 cells.[2] Canonne-Hergeaux et al analyzed expression of Nramp2 in the duodenal epithelium of *mk* mice and determined that the mutant protein did not target to the cell surface.[3] In this issue of *Blood*, Touret and colleagues carefully examined these and other possible mechanisms by which the G185R is defective. They stably expressed in HEK293 cells one of the Nramp2 isoforms and the corresponding G185R mutant protein. The investigators found that although wild-type Nramp2 exists primarily as a "complex-glycosylated" protein in the plasmalemma, the G185R Nramp2 protein exists primarily as a "core-glycosylated" precursor in the endoplasmic reticulum. This mutant Nramp2 precursor is subject to rapid degradation by a proteosome-dependent mechanism. Although a small fraction of the G185 mutant Nramp2 is fully glycosylated and traffics to the plasmalemma, this fraction has a shortened half-life. The activity of this residual fraction, after normalizing for the level of expression at the cell surface, was found to be only 60% of wild-type Nramp2.



Subcellular distribution of Nramp2^{G185R}. See the complete figure in the article beginning on page 1526.

There are only a few limitations to this extensive body of work. Only 1 of the 4 different Nramp2 isoforms was studied, and it is possible that another isoform of the mutant protein may be processed to a different degree. Furthermore, marked overexpression of the mutant protein in these studies may have overwhelmed cellular compensatory mechanisms and exaggerated abnormalities in processing and/or trafficking. Finally, it is possible that cellular processing of the mutant protein may be different in the cell types in which Nramp2 expression is functionally most important (eg, polarized duodenal mucosal cells and erythroid precursor cells). It is unlikely, however, that any of these issues challenge the most important observations: (1) markedly decreased expression of G185R Nramp2 on the plasmalemma; and (2) modest decrease in transport activity.

The authors propose a model in which abnormal folding of the mutant protein promotes the rapid proteosomal degredation and downstream events. If this model is correct, these deficits might be partially overcome by the use of chemical chaperones. In summary, the Nramp2 G185R protein was found to demonstrate multiple biosynthetic and functional deficits: abnormal processing, increased degradation, mislocalization, and decreased transport activity. Through their careful analysis, Touret and colleagues were able to corroborate 2 studies with potentially disparate findings and add important new observations to explain the functional deficits of G185R Nramp2. ■

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RED CELLS

Comment on Chou et al, page 1498

Is there something fishy about EPO?

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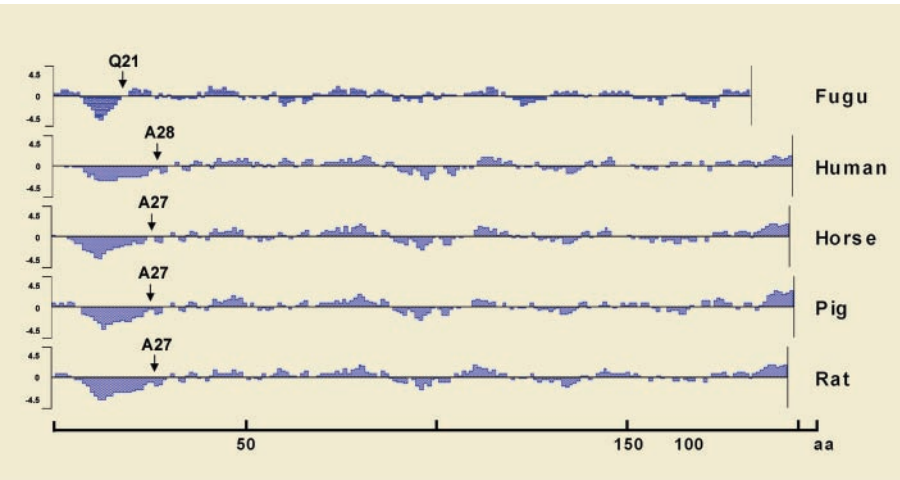
The erythropoietin gene from *Fugu rubripes* (pufferfish) is homologous to the human protein but lacks a flanking hypoxia-responsive element that is critical for erythropoietin induction by hypoxia in mammals.

Work by Chou and colleagues in this issue represents the first study of a nonmammalian erythropoietin (*Epo*) gene in the gourmet's fish delicacy, *Fugu rubripes* (pufferfish). Using homology with the human EPO protein sequence, the teleost *Epo* gene was identified in silico from the Fugu genome, indicating similar exon organization but only 32% homology with the human protein.

Hypoxic *EPO* gene induction in mammals is regulated by a hypoxic response element (HRE) in the 3′ untranslated region (UTR) that binds hypoxia-inducible factor 1 (HIF-1).[1] Hypoxia increases HIF-1α stability allowing for nuclear localization, dimerization with HIF-1β to form HIF-1, and transcription activation via HRE binding. The Fugu *Epo* gene has no functional HRE in the 5′ or 3′ flanking regions, although there is some indication that hypoxia increases appropriate splicing of *Epo* transcripts. This altered response to hypoxia may reflect the low oxygen availability in the aquatic environment and a significant difference in regulation of erythropoiesis between mammals and fish, or the limitation of available reagents, and suggests important areas for further investigation.

An important evolutionary development in vertebrates is the delivery of oxygen by hemoglobin-encapsulated erythrocytes. Much is known about hypoxia induction of EPO in the kidney and EPO stimulation of bone marrow erythropoiesis in mammals, especially humans and rodents. In contrast, Fugu *Epo* is expressed mainly in the heart, with some expression in brain and liver, but not in kidney, and erythropoiesis takes place in the kidney rather than in the bone marrow. Nevertheless, it appears that *Epo* synthesis away from the site of erythropoiesis and its secretion into the circulation for transport to stimulate red cell production is conserved between mammals and fish. Of note is the similarity of *Epo* expression in brain and liver. In mammalian model systems, EPO effects extend beyond erythropoiesis and provide protection in the embryo and select adult organs against ischemia or stress. In addition to EPO requirement for erythropoiesis, we observed a developmental defect in brain neuroepithelium and in heart endocardium and myocardium as well as increased neuron sensitivity to hypoxia in mice that lack the erythropoietin receptor.[2] Neuroprotection by EPO has been demonstrated in several adult animal models for brain hypoxia and mechanical trauma and includes EPO protection against brain ischemia that reduces hippocampal neuron damage and memory loss.[3] The high level of *Epo* expression in the Fugu heart report by Chou and colleagues also draws attention to recent reports of EPO protection in mammalian models of heart ischemia. For example, a single dose of EPO following myocardial infarction in rats reduced infarct size and functional decline 8 weeks after insult.[4] Fugu *Epo* expression in heart and brain raises the possibility that its neuroprotective and cardioprotective activities require local EPO production, may be evolutionarily conserved, and perhaps were among the original functions of this molecule. Chou and colleagues point out important similarities and differences between mammalian and Fugu *Epo*. Further understanding of the spatial and temporal expression of teleost *Epo* may yield important insight into vertebrate erythropoiesis and EPO function in other tissues. ■



The hydrophilicity plots for Epos from Fugu, human, horse, pig, and rat generated using Kyte & Doolittle hydrophilicity parameters. See the complete figure in the article beginning on page 1498.

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