REDOX PIONEER #13

Redox Pioneer: Professor Vadim N. Gladyshev

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Professor Vadim N. Gladyshev

Abstract

Professor Vadim N. Gladyshev is recognized here as a Redox Pioneer, because he has published an article on antioxidant/redox biology that has been cited more than 1000 times and 29 articles that have been cited more than 100 times. Gladyshev is world renowned for his characterization of the human selenoproteome encoded by 25 genes, identification of the majority of known selenoprotein genes in the three domains of life, and discoveries related to thiol oxidoreductases and mechanisms of redox control. Gladyshev's first faculty position was in the Department of Biochemistry, the University of Nebraska. There, he was a Charles Bessey Professor and Director of the Redox Biology Center. He then moved to the Department of Medicine at Brigham and Women's Hospital, Harvard Medical School, where he is Professor of Medicine and Director of the Center for Redox Medicine. His discoveries in redox biology relate to selenoenzymes, such as methionine sulfoxide reductases and thioredoxin reductases, and various thiol oxidoreductases. He is responsible for the genome-wide identification of catalytic redox-active cysteines and for advancing our understanding of the general use of cysteines by proteins. In addition, Gladyshev has characterized hydrogen peroxide metabolism and signaling and regulation of protein function by methionine-*R*-sulfoxidation. He has also made important contributions in the areas of aging and lifespan control and pioneered applications of comparative genomics in redox biology, selenium biology, and aging. Gladyshev's discoveries have had a profound impact on redox biology and the role of redox control in health and disease. He is a true Redox Pioneer. *Antioxid. Redox Signal.* 25, 1–9.

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The greatest discoveries in science still lie ahead.

—Prof. Vadim N. Gladyshev

Educational and Professional Training

DR. GLADYSHEV OBTAINED his education at Moscow State University, Moscow, Russia. He did his postdoctoral training with Dr. Thressa Stadtman, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), and then with the author, National Cancer Institute (NCI). His first faculty appointment was in the Department of Biochemistry, the University of Nebraska, Lincoln, NE. Dr. Gladyshev became a full professor in only 6 years and then received a

Charles Bessey Professorship. He served as the Director of the Redox Biology Center until 2009, when he moved to the Department of Medicine, Brigham, and Women's Hospital, Harvard Medical School, where he is Professor of Medicine and Director of the Center for Redox Medicine.

Background, Development, and Training

Vadim Gladyshev was born in 1966 and was raised in Orenburg, the Union of Soviet Socialist Republics. He

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Author note: Vadim Gladyshev spent 15 months in the author's laboratory as a postdoctoral fellow prior to his faculty appointments. The author and Professor Gladyshev have closely collaborated in publishing more than 100 papers jointly.

For a list of frequently cited articles published by Prof. Vadim Gladyshev, see Supplementary Tables S1 and S2, available online at www.liebertpub.com/ars

Vadim N. Gladyshev (*right*) with the author (December 2015)

graduated from high school with the highest honors (gold medal), while also completing music school. He credits his high school teacher, Maria Kashkareva, for stimulating his interest in chemistry and introducing him to experimental and current science. Her emphasis on excellence contributed heavily to Gladyshev receiving the first prize in chemistry in the state (Orenburg region) twice during his high school years. His father was an engineer, and his mother worked for a local newspaper. They provided him unconditional support and instilled in him to achieve the best education possible.

In 1983, Gladyshev was accepted to Moscow State University, the finest university in the Union of Soviet Socialist Republics, where he received BS/MS degrees with the highest honors (red diploma) in 1988 and a doctorate in biochemistry in 1992. He started doing research at the age of 18, joining a lab at the Institute of Biochemistry, Russian Academy of Sciences. He played chess throughout his college years for his university and department (being captain), achieving the level of national master.

For postdoctoral training, Gladyshev joined the Laboratory of Biochemistry, NHLBI, NIH, working with Thressa Stadtman, who had previously discovered that the trace element selenium was present in protein in the form of selenocysteine (Sec) (9, 55). The superb scientific environment in the NHLBI was instrumental in Gladyshev's early training, and it played a significant role in his future scientific endeavors.

At the NHLBI, Gladyshev used electron paramagnetic resonance spectroscopy and other biophysical methods to show that selenium was coordinated to molybdenum in the active site of nicotinic acid hydroxylase (19). This established a function for selenium as a cofactor that did not involve Sec. He also demonstrated the coordination of Sec to molybdenum in formate dehydrogenase H (20). Gladyshev spent 2 years crystallizing this enzyme. A challenge was that it was highly sensitive to molecular oxygen. He spent many hours in the NHLBI Anaerobic Laboratory, the only such facility in the world (51). After more than a year of failed crystallization attempts, he found a beautiful crystal and, in collaboration with Peter Sun of the National Institute of Allergy and Infectious Diseases, the structure of formate dehydrogenase was solved (6).

While initially working with bacterial selenoproteins, Gladyshev became interested in the role of selenium in

mammalian cells. This led to a collaboration with Kuan-Teh Jeang. Although they found that HIV did not code for selenoproteins (23), both infected and uninfected T cells exhibited a complex pattern of selenoproteins, which was also noted by other groups (3). This observation led Gladyshev to isolating and characterizing various selenoproteins. The first was thioredoxin reductase (1). A selenoprotein with thioredoxin reductase activity was previously detected (60), but it did not react with the thioredoxin reductase antibody. The human thioredoxin reductase gene had been cloned (17), but it was not recognized as a selenoprotein. Gladyshev succeeded in isolating thioredoxin reductase and sequencing its ⁷⁵Se-labeled peptide, wherein the selenium moiety corresponded to Sec encoded by UGA in the C-terminal penultimate position (21). This finding established the dependence of the mammalian thioredoxin system on selenium.

In 1996, Gladyshev moved to the Basic Research Laboratory, NCI, receiving training in cell and molecular biology with the author. There, he discovered another selenoprotein, the 15 kDa selenoprotein (Sep15) (22). While analyzing its sequence, he noticed significant variation among mouse Sep15 sequences. His curiosity led to the discovery that the major mouse cDNA library used by the research community was actually derived from rats. He contacted the National Center for Biotechnology Information, who then took action to correct it. Shared scientific interests of the author and Gladyshev developed during his postdoctoral training, resulting in a long-lasting collaboration and friendship. Together, they published 112 research articles and, in addition, have written numerous book chapters and edited three books.

In 1998, Gladyshev joined the Department of Biochemistry, the University of Nebraska-Lincoln, as an assistant professor. He quickly rose through the academic ranks and became a full professor after only 6 years. In 2005, he was named Charles Bessey Professor of Biochemistry and, in 2007, he became Director of the Redox Biology Center. In 2008, Gladyshev received the Outstanding Research and Creative Activity award, the highest recognition in the University of Nebraska system.

In 2009, Gladyshev moved his laboratory to the Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, where he remains as Professor of Medicine and Director of the Center for Redox Medicine. He is also an Associate Member of the Broad Institute of Harvard and MIT, and a faculty member of the Dana Farber Cancer Center. In recent years, he has also been a visiting Distinguished Professor at Ewha Womans University in Seoul, Korea (2010–2013), and a Founding Faculty Fellow of the Skoltech Institute of Science and Technology, Moscow, Russia (2011–2015).

In 2006, Gladyshev established and chaired a new Gordon Research Conference on "Thiol-based Redox Regulation and Signaling." This premier antioxidant/redox biology conference is currently a regular and very successful meeting. Gladyshev also chaired an FASEB summer conference and several other meetings. Gladyshev was elected to AAAS and is the recipient of EUREKA, MERIT, and PIONEER awards from NIH.

Areas of Interest in Redox Biology

The human selenoproteome

Selenoproteins were discovered in the early 1970s (12, 52) and are key redox regulators in cells due to the presence of

catalytic redox-active Sec residues in these proteins. However, at the beginning of Gladyshev's faculty career, less than 10 mammalian selenoproteins were known. The beneficial effects of dietary selenium in nutrition and health were largely attributed to the occurrence of selenium in these proteins (55). However, there was an important question that needed to be resolved: How many selenoproteins exist in humans? Completion of the human genome sequence was of little help in resolving this question, as the UGA codons that encoded Sec were interpreted as stop signals. How then could selenoprotein genes be identified?

Gladyshev's solution was to identify selenoprotein genes by searching for Sec insertion sequence (SECIS) elements, which are stem-loop structures in the 3'-UTRs of selenoprotein genes (Fig. 1). This element was previously discovered by Marla Berry in deiodinase (4). Since the primary sequence of these elements was not conserved, the approach that Gladyshev developed required analyses of structural RNA elements, calculation of the free energy for various parts of the candidate SECIS elements, and examination of these structures across species to detect those that are conserved and flanked by less conserved sequences.

Gladyshev's group (33) and the group of Alain Krol (42) first applied this strategy to analyses of expressed sequences, revealing new selenoproteins (SelR, SelT, and SelN). Then, genome-wide analyses, carried out by Gladyshev's graduate student, Gregory Kryukov, and in collaboration with the group of Roderic Guigo, yielded 25 selenoprotein genes, comprising the entire human selenoproteome (35). This approach represented the first example of human gene discovery based on searches for RNA structures. It worked so well that all genes identified were confirmed as selenoproteins and not a single additional human selenoprotein has subsequently been identified.

The fact that human selenoproteins with known functions (glutathione peroxidases, thioredoxin reductases, and thyroid hormone deiodinases) were oxidoreductases suggested that at least some, if not all, additional selenoproteins also possessed redox functions. Indeed, more than half of the human selenoproteome exhibited thioredoxin folds. Most importantly, information on the entire set of selenoproteins directly linked

selenium with specific biological functions. In addition, since the human selenoproteome consists of proteins encoded by only 25 genes, the system as a whole became amenable to genetic manipulations, and this is an area in which the author and Gladyshev published ~50 papers. The human selenoproteome study led by Gladyshev is of profound importance. This work was published in *Science* in 2003 (35) and has since been the most cited paper in the field.

Discovery of selenoproteins in the three domains of life and components of Sec biosynthesis and insights into the genetic code

In addition to the human selenoproteome, the Gladyshev lab has discovered the majority of all known selenoprotein genes in the three domains of life (Fig. 2) (27). It is now known that the zebrafish genome codes for 39 selenoprotein genes, mouse 24, and *Drosophila melanogaster* 3 (37). *Caenorhabditis elegans* has only a single Sec (in thioredoxin reductase) encoded in its genome, whereas the budding yeast genome lacks selenoprotein genes. These findings linked dietary selenium with the genes in model organisms that mediate its effects in biology and health and led to the discovery of many new functions for selenium and selenoproteins.

Gladyshev has published more than 50 papers characterizing various selenoproteomes across the tree of life. For example, he found that anthropomorphic selenium is responsible for brown tides due to dependence of the corresponding algae on its large selenoproteome (26), that aquatic life is associated with increased utilization of selenium, that terrestrial life manifests decreased use of this element (43), and that mammals exhibit a reduced utilization of selenium during evolution (44).

Due to Gladyshev and his colleagues' identifying the selenoproteins in virtually all organisms whose genomes have been sequenced, it is known that 80% of prokaryotes lack the ability to incorporate selenium into protein as Sec, whereas 50% of eukaryotes utilize Sec (65). He extended these approaches to copper, molybdenum, cobalt, and nickel, providing insights into the biology of these elements (66).

Gladyshev used comparative genomics approaches to discover two components involved in the biosynthesis of Sec, phosphoseryl-tRNA kinase (8), and Sec synthase (63). This allowed his and the author's laboratories to characterize the entire pathway of Sec biosynthesis in eukaryotes (63) (Fig. 3), which was previously known only in bacteria (5). The discovery of phosphoseryl-tRNA kinase was especially instructive, as the search targeted kinases that are present exclusively in those archaea and eukaryotes that also possess selenoprotein genes. The discovery of the two enzymes involved in Sec biosynthesis was instructive, because no other amino acid is synthesized exclusively on a tRNA scaffold in eukaryotes and because bacteria use a completely different enzyme to synthesize Sec (5).

The scholarly approach of using comparative genomics, systems biology, and high-throughput methods, and combining them with biochemical and genetic approaches, has repeatedly demonstrated Gladyshev's ingenuity. He extended this strategy to better understand pyrrolysine (Pyl, the 22nd amino acid, genetically encoded by a UAG codon). He showed that Sec and Pyl use dissimilar decoding strategies, providing additional insights into the decoding of rare amino

FIG. 1. Identification of selenoprotein genes. Searches for SECIS elements was the strategy used to identify selenoprotein genes in sequence databases. Searches for Sec/Cys pairs in homologous sequences identified both selenoproteins and thiol oxidoreductases. Sec aligned with Cys the indicated location of catalytic redox-active Cys residues. SECIS, Sec insertion sequence.

FIG. 2. Selenoprotein families. The figure lists major selenoprotein families. Relative lengths of selenoprotein sequences are indicated on the *right*, with the location of Sec shown by a *vertical mark*. Selenoproteins shown in *bold* are found in prokaryotes, and those *boxed* are seen in eukaryotes (separately for single-celled eukaryotes and vertebrates). Some selenoproteins are present in both prokaryotes and eukaryotes. *Star* (*) after the selenoprotein name indicates that the protein was first described by Gladyshev. The majority of selenoproteins have orthologs with Cys in the position corresponding to Sec, and some Cys-containing forms are dominant (*e.g.*, thioredoxin, glutaredoxin).

acids (64), and also found that additional amino acids as abundant as Sec do not exist (44a). Another highly significant study from the Gladyshev lab was the discovery that one codon in an organism can code for two different amino acids, even within the same gene. He found that *Euplotes crassus* uses UGA to code for cysteine (Cys) and Sec (62). This study represents a major contribution in expanding our understanding of the genetic code.

Selenoprotein redox functions

After the discovery of selenoproteins, Gladyshev's lab characterized many of these oxidoreductases (Fig. 2). One such protein was thioredoxin-glutathione reductase, a mammalian protein with specificity for both the thioredoxin and glutathione

systems due to the fusion of thioredoxin reductase and glutaredoxin domains (57, 58). Gladyshev further identified the role of this protein in maintenance of disulfide bonds during sperm maturation (56). He was one of the first to report on the mitochondrial thioredoxin reductase (18, 38, 57) and to characterize multiple forms of mammalian thioredoxin reductases (59).

One of the mammalian selenoproteins discovered by Gladyshev was initially designated as Selenoprotein R (SelR) (33). Insights into the function of this protein came from the observation that, in some bacteria, SelR was fused to methionine sulfoxide reductase A (MsrA), and/or thioredoxin and glutaredoxin (34), and that the genes for these proteins were often located in the same operon. This firmly linked SelR to the process of methionine sulfoxide reduction, a process previously extensively characterized by Herbert Weissbach and Earl

FIG. 3. Biosynthesis of Sec. Sec is synthesized on its tRNA. Two proteins involved in this process (PSTK and SEPSECS) were identified by the Gladyshev lab. Model organisms are shown to illustrate the use of powerful tools of comparative genomics to identify and functionally characterize selenoproteins and components of Sec biosynthesis. Organisms illustrated are clockwise from the top: archaea, nematode, fruit fly, and mouse.

Stadtman (7, 50). Subsequent biochemical assays by the Gladyshev lab revealed that this protein was indeed a methionine sulfoxide reductase, and it was renamed as methionine sulfoxide reductase B (MsrB). Interestingly, MsrB is specific for the *R* stereoisomer of methionine sulfoxide (34), whereas MsrA is specific for the *S* stereoisomer. The Gladyshev lab reported on differences in catalytic mechanisms between selenoprotein methionine sulfoxide reductases and their Cys-containing orthologs (31) and characterized numerous members of the MsrB and MsrA families of enzymes, including all such proteins in mammals (30).

Since MsrA and MsrB differentially reduce methylsulfoxides in drugs, these enzymes have been implicated in drug efficacy (39). Other studies from the Gladyshev lab used methionine sulfoxide reduction to reveal reactive oxygen species (ROS)-dependent and -independent components of aging (36), to identify methionine restriction as a general strategy for lifespan control (41), and to develop fluorescent ratiometric sensors for *in vivo* monitoring of methionine sulfoxide (61). They also recently discovered a new exciting mechanism of regulation of protein function: reversible, site-specific methionine-*R*-sulfoxidation (40). Here, an oxidoreductase called Mical oxidizes actin on two methionine residues to methionine-*R*-sulfoxide (promoting actin depolymerization), whereas MsrB reduces them back to methionine (promoting polymerization). In mammals, the primary MsrB involved in this process is the selenoprotein, MsrB1.

Mechanisms of thiol-based redox control

In selenoproteins with known functions, Sec is located in the active sites and serves catalytic redox functions. In orthologous Cys-containing proteins, Cys is the corresponding redox-active residue (Fig. 4). However, a Sec residue immediately implicates a redox function (and specifies location of the redox group); whereas a Cys residue, in addition to redox, may have many other functions, for example, it may form disulfide bonds, coordinate metal ions, be post-translationally modified, and serve nonredox functions in enzyme active sites. How could this relationship between Sec and Cys be explored to identify redox-active Cys residues? Gladyshev posited that the comparative genomics approach would yield Sec-Cys pairs in homologous sequences, wherein Sec aligned with a Cys can indicate a redox function of that Cys and identify the corresponding protein as a thiol oxidoreductase.

In addition, by analogy with Sec, the newly identified redox-active Cys should correspond to nucleophilic Cys residues rather than resolving Cys residues in the oxidoreductase. Using this approach, Gladyshev developed a method to predict catalytic redox-active Cys residues on a genome-wide scale, verifying known thiol oxidoreductases and leading to the identification of many new such proteins, some of which have been experimentally characterized (13). This method also allowed defining thiol oxidoreductases as a

Selenocysteine versus Cysteine

FIG. 4. Sec and Cys. The figure compares Sec and Cys residues in proteins. They are encoded by different codons. Free Sec is distinguished from Cys by its lower pKa. Both residues can catalyze the same reactions (*e.g.*, reduction of a disulfide bond, as shown in the figure). However, Cys has various functions (representative functions are listed in the figure), whereas Sec is used primarily for redox catalysis, that is, Sec is the catalytic redox-active residue in proteins. Therefore, Sec may be viewed as "supercysteine."

group, which was found to represent $\sim 1\%$ of the proteome in the organisms examined (14). This research revealed that thiol-based redox regulation is present in each of these organisms. Thus, this type of redox control is essential to life!

Another major insight from the Gladyshev lab relates to the utilization of Cys residues in proteins. Commonly used hydrophobicity scales report Cys as being hydrophobic based on its frequent occurrence in buried areas of proteins. How-

ever, the chemical nature of Cys is clearly inconsistent with this definition. The Gladyshev lab found that Cys residues appear buried, because many such residues are removed from protein surfaces during evolution. In his model, Cys is more reactive and, therefore, slightly more deleterious than other residues, especially when it is exposed (49). Thus, many and perhaps the majority of surface-exposed Cys are functional! Gladyshev has also published several studies on regulatory redox-active Cys. His protein structure analyses revealed that trans-nitrosylation may be a major mechanism of Cys S-nitrosylation (48).

Gladyshev also published extensively on thiol oxidoreductases, for example, being among the first to report on glutaredoxin 2 (24, 45), and has contributed significantly to the areas of hydrogen peroxide metabolism and redox signaling. Thiol peroxidases (*i.e.*, glutathione peroxidase and peroxiredoxins) are known to remove hydroperoxides. After the discovery that a glutathione peroxidase was required for activation of the Yap1 transcription factor (10), Gladyshev reported that thiol peroxidases mediate the effects of hydrogen peroxide in redox signaling (15). His lab discovered that the mutant lacking all such proteins in yeast was unable to sense hydrogen peroxide. Thus, thiol peroxidases transfer oxidizing equivalents, and this process largely controls redox signaling through hydrogen peroxide, whereas direct oxidation of Cys residues by this oxidant plays a secondary role in this process.

A further advance in this area was the discovery that adaptive aneuploidy can protect against oxidative stress (29). Gladyshev found that, under conditions of thiol peroxidase deficiency, yeast cells duplicate one of its chromosomes to stimulate mitochondrial function, which, in turn, provides reducing equivalents from the electron transport chain to reduce hydrogen peroxide (29). Although mitochondria are often viewed as the source of ROS, this study showed that mitochondria were essential for removing hydrogen peroxide in yeast.

FIG. 5. Research on long-lived mammals. The Gladyshev lab has sequenced the genomes and/or transcriptomes of several exceptionally long-lived organisms. Gladyshev is shown holding a naked mole rat. The *bottom panel* shows (*left to right*) the Brandt's bat, bowhead whale, naked mole rat, and Damaraland mole rat (their lifespan and body weights are listed on the *right side* of the figure).

Other Achievements and Current Position

Gladyshev has contributed significantly to several other areas of science, publishing altogether ~300 papers. His current h-factor is 78 according to Google Scholar and 62 according to the ISI Web of Science. The author will mention, although briefly, Gladyshev's contributions to the area of aging, including mechanisms of aging and control of lifespan, in addition to the application of redox biology to understanding aging discussed earlier. The Gladyshev lab also sequenced and characterized the genomes, transcriptomes, and/or metabolomes of several exceptionally long-lived mammals, most notably the naked mole rat, Brandt's bat, and bowhead whale (11, 32, 53, 54), providing numerous insights into the biology of these animals, their adaptations, and the associated molecular mechanisms involved (many of which were related to selenium and redox biology) (Fig. 5). He also identified longevity signatures based on gene expression, metabolite profiles, and elemental profiles (16, 46, 47), providing information on how lifespan is shaped during evolution and how this information can be used to adjust species lifespan. The naked mole rat genome was the first genome sequenced with the goal of understanding longevity. He has published extensively on mechanisms of aging, offering new concepts that define aging and the evolution of lifespan (2, 25, 28).

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Abbreviations Used

NCI = National Cancer Institute
 NHLBI = National Heart, Lung, and Blood Institute
 NIH = National Institutes of Health
 ROS = reactive oxygen species
 SECIS = Sec insertion sequence