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Redox Pioneer: Professor Barry Halliwell

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Professor Barry Halliwell

Abstract

Professor Barry Halliwell is recognized as a Redox Pioneer because he has published eight articles on redox biology that have been each cited more than 1000 times, and 158 articles that have been each cited more than 100 times. His contributions go back as far as 1976, when he was involved in elucidation of the Foyer-Halliwell-Asada cycle, an efficient mechanism for preventing oxidative damage to chloroplasts. His subsequent work established the important role of iron and zinc in free radical reactions and their relevance to human pathologies. Professor Halliwell is also a leader in developing novel methodology for detecting free radical intermediates *in vivo*, and his contributions to our knowledge of reactive nitrogen species are highly significant. His sustained excellence won him the top-cited scientist award in the United Kingdom in biomedical sciences in 1999, and in 2003 he was recognized as a highly cited scientist by Institute of Scientific Information (ISI) for work on plant antioxidants, and the same year ranked 28 out of 5494 biochemists/biologists for scientific impact. Two pieces of his scholarly work have been listed as Citation Classics by ISI, and in 2007 his laboratory was ranked number 1 worldwide based on highest citation score in research on free radicals. *Antioxid. Redox Signal.* 14, 1761–1766.

> I got into the field of free radicals and antioxidants by following up a chance observation I made during my Ph.D. work. At the time, few were interested in redox biology. Now everyone is. Don't be afraid to take a risk and explore a sudden inspiration or a new concept. You may help create a new research field and then many will follow.
>
> —Professor Barry Halliwell

Educational and Professional Training

PROFESSOR HALLIWELL OBTAINED his B.A. and D.Phil. degrees from the University of Oxford, and a Doctor of Science (D.Sc.) from the University of London, England. He did postdoctoral training in Biochemistry at St. Cross College, Oxford, lectureship and readership in Biochemistry at the University of London, King's College, and a fellowship in Preventive Medicine sponsored by the Lister Institute, Bushy, England.

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Author note: Shazib Pervaiz is a full Professor in the Department of Physiology at the Yong Loo Lin School of Medicine, National University of Singapore, and in the Cancer and Stem Cell Biology Program, Duke-NUS Graduate Medical School, Singapore. He has followed Professor Halliwell's work since his own days as a graduate student, and served on a number of scientific bodies jointly with Professor Halliwell.
For a list of frequently cited articles published by Prof. Halliwell, *see* Supplementary Tables S1 and S2, available online at www.liebertonline.com/ars.

Summary of Professor Halliwell's Top Scientific Contributions

Professor Halliwell's first major contribution goes back to 1976, when he elucidated the ascorbate-glutathione cycle, now often called the Foyer-Halliwell-Asada cycle, an efficient mechanism in plants for scavenging peroxides to protect the chloroplast against damage. He is also recognized for his pioneering work in establishing the important role of transition metal ions, in particular "mal-placed iron" and zinc, in free radical reactions and their relevance to human disease states. His work has unraveled the pattern of oxidative damage and the free radical species involved in Parkinson's and Alzheimer's disease. Professor Halliwell has also been at the forefront of developing novel methodology for detecting free radical intermediates *in vivo* and designing techniques for characterizing antioxidants. In addition, his contributions to our knowledge of reactive nitrogen species such as peroxynitrite are also highly significant. Last but not least, the textbook *Free Radicals in Biology and Medicine* authored by Professors Halliwell and John Gutteridge is the most comprehensive resource on the subject and considered by many in the field as the Bible of free radical chemistry and biology.

Description of Key Finding 1

Discovery of the Foyer-Halliwell-Asada cycle

The integrity of photosynthesis in plants is dependent on the redox state of chloroplasts, and therefore in an oxygen-rich environment it is imperative to limit the level of exposure of chloroplast to superoxide radical ($O_2^{\bullet -}$) and its dismutation product hydrogen peroxide (H_2O_2). To that end, Professor Halliwell pioneered the discovery of a novel pathway for maintaining the functional state of the chloroplasts (6). This pathway or cycle (Fig. 1) is referred to as the Foyer-Halliwell-Asada cycle after the names of the three major contributors. It is also known as the ascorbate-glutathione cycle or the water–water cycle. Halliwell, Asada, and others identified three major components in this pathway for the removal of H_2O_2. As chloroplasts lack catalase, a natural scavenger of H_2O_2, they rely on the reduction of H_2O_2 to H_2O *via* ascorbate-specific peroxidases (reaction 1) using ascorbate as a reductant and in the process generating a one-electron oxidation product of ascorbate, monodehydroascorbate radical. The latter is reduced back to ascorbate in the presence of dehydroascorbate reductase (reaction 2) together with the oxidation of GSH (reduced glutathione) to GSSG (oxidized glutathione). In the final step of the cycle, glutathione reductase (reaction 3) regenerates GSH from GSSG with the oxidation of NADPH to $NADP^+$. This was truly an important discovery as it clearly highlighted the deleterious effects of reactive oxygen species on enzymes involved during photosynthesis, such as the biphosphatases, and underscored the importance of maintaining a reducing environment for efficient photosynthesis.

Description of Key Finding 2

Metal-catalyzed radical reactions and human disease

Professor Halliwell is also recognized as one of the fundamental pioneers in establishing the important role of transition metal ions in catalyzing free radical reactions *in vitro*. He and colleagues showed that whereas iron bound to lactoferrin and transferrin is generally unable to catalyze free radical reactions at physiological pH (2), a limited amount of iron can be released from other sources by oxidative stress (22). Iron catalytic for free radical reactions thus appears to be available within cells. Hence, an important function of intracellular antioxidant defense enzymes is to scavenge $O_2^{\bullet -}$ and H_2O_2 before they can come into contact with this available iron (10, 21). He followed up by demonstrating that these reactions also occurred *in vivo*; the small pool of non-protein-bound iron moving between transferrin, cell cytoplasm, mitochondria, and ferritin could provide iron to enter into the Fenton reaction generating the highly reactive hydroxyl radical ($^{\cdot}OH$). In particular, work from his group highlighted the importance of "mal-placed" iron and its ability to promote oxidative damage in several human pathologies, including iron overload disease, cardiac damage, cancer chemotherapy-induced toxicity, inflammatory disorders such as rheumatoid arthritis, and vascular pathologies such as atherosclerosis (8, 22, 30, 32). His recent studies on atherosclerosis again underscore the role of iron in disease promotion and additionally show a significant decrease in atherosclerotic lesion formation by zinc, at least in rabbits (28, 29).

Description of Key Finding 3

Detection of free radicals in vivo *and radical-induced damage in disease states*

For decades, one of the issues confronting researchers and clinicians in relation to the impact of redox reactions on

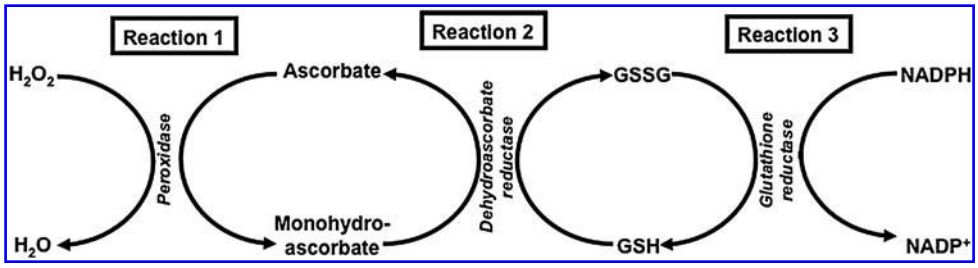

FIG. 1. Foyer-Halliwell-Asada Cycle. Chloroplasts lack catalase, and rely on the reduction of H_2O_2 to H_2O *via* ascorbate-specific peroxidases (reaction 1) using ascorbate as a reductant and in the process generating a one-electron oxidation product of ascorbate, monodehydroascorbate radical. The latter is reduced back to ascorbate in the presence of dehydroascorbate reductase (reaction 2) together with the oxidation of GSH (reduced glutathione) to GSSG (oxidized glutathione). In the final step of the cycle, glutathione reductase (reaction 3) regenerates GSH from GSSG with the oxidation of NADPH to $NADP^+$.

Professor Barry Halliwell's group at the National University of Singapore, Singapore.

human physiology and pathology has been the relative lack of stringent methodology to measure free radicals and their related species *in vivo*. Professor Halliwell and his group were among the first to recognize this problem and were at the forefront of designing novel methodology for the *in vivo* detection and quantitation of free radical species. These methods were based on aromatic hydroxylation (9, 11, 20, 28–30, 32), urate degradation (9), and the measurement of chemical fingerprints of DNA damage caused by free radical attack (19). The latter involved collaborative endeavors with experts on mass spectrometry to obtain a detailed characterization of the pattern of DNA damage caused by different free radicals and related species (19). This novel methodology was then put into use to decipher and delineate the pattern of oxidative damage in primary brain tissue from patients with Parkinson's and Alzheimer's disease, thus helping to identify the damaging free radical species *in vivo* (1, 16, 27, 31). The same strategy provided insights into the mechanism (s) and significance of oxidative DNA damage in cancer development and in Parkinson's disease (1, 18). Other work on Parkinson's disease characterized the ready formation and cytotoxicity of quinones (31). As an extension of this work, Professor Halliwell has made a significant contribution to the concept that proteasomal dysfunction is closely linked to oxidative damage and neuronal death in neurodegenerative disorders (17) (summarized in Fig. 2), which could have tremendous therapeutic implications (15, 16, 26).

Other Achievements and Contributions

Reactive nitrogen species in human health and disease

In addition to his valuable contributions in the area of oxidative stress, Professor Halliwell has also made substantial contributions to our knowledge of reactive nitrogen species such as peroxynitrite, often assayed *in vivo* by measuring the nitration of tyrosine residues. He and Kevin Moore pioneered the development of one of the first techniques, based on mass spectrometry, which allows nitrotyrosine to be measured accurately in human tissues and body fluids (7). In an interesting set of collaborative studies with Eiserich, Cross, van der Vliet, *et al.*, it was demonstrated that much nitration *in vivo* might not be a function of peroxynitrite production, but involve myeloperoxidase-dependent reactions (5, 13, 33). As a logical progression of these studies, subsequent work from Halliwell's group showed that nitrative damage to proteins, oxidative stress, and proteasomal impairment could be part of the same series of neurotoxic events in the brain during neurodegenerative disease (1, 15, 16). Barry also contributed significantly to fundamental studies of the mechanisms by which oxidizing air pollutants (O_3, NO_2) can interact with human body fluids (3, 4).

Antioxidants and nutrition: myth or reality?

Professor Halliwell has also made major contributions over the years in the field of molecular nutrition by way of direct measurements of oxidative damage in the human body. His work has been instrumental in dispelling the notion that vitamins C, E, and β-carotene provide significant health benefits as antioxidants by providing evidence that these agents do not generally decrease oxidative damage *in vivo* (14). This helps to explain why many of these antioxidants are showing limited efficacy in human intervention trials testing for disease prevention. His laboratory is now identifying the most important antioxidants in natural foodstuffs, such as dark soya sauce, flavonoids, and tocotrienols, and examining their effects *in vivo* (25, 34). He has also been at the forefront of developing techniques for characterizing antioxidants and examining their effects *in vitro* and *in vivo*, which are now widely used (12, 14). Further, Professor Halliwell also pioneered the concept that antioxidants may exert many of their effects within the gastrointestinal tract, and that antioxidants can sometimes exert benefit by acting as pro-oxidants *in vivo* (23, 24).

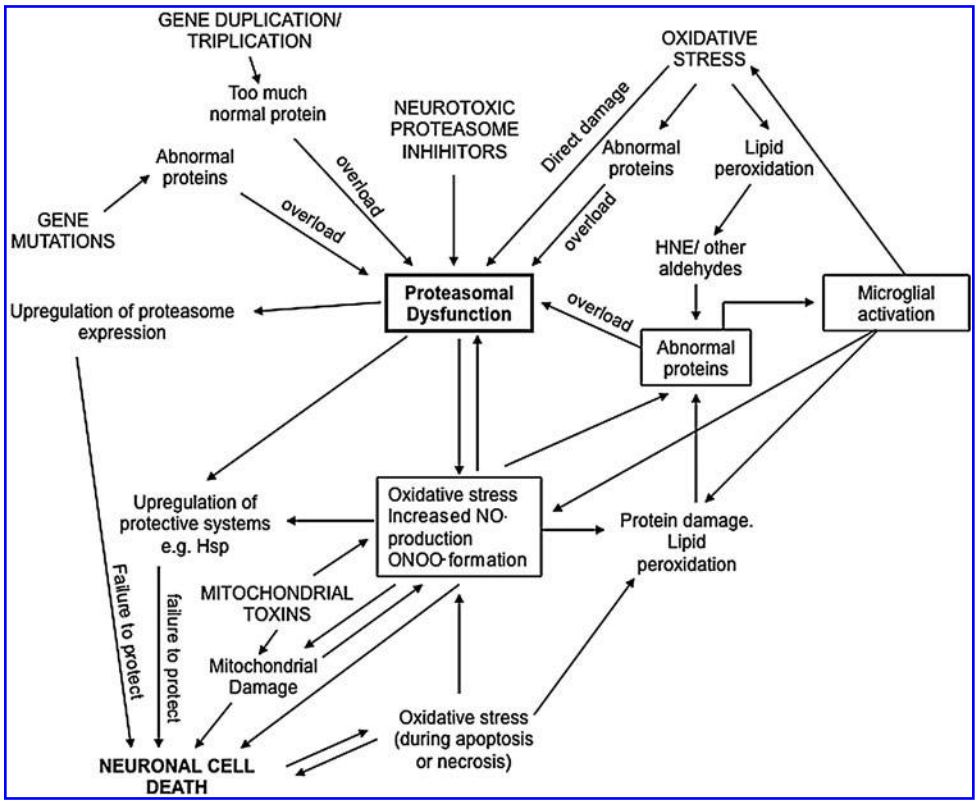

FIG. 2. A unifying hypothesis: Interplay of mitochondria, oxidative damage, and proteasome in neurodegeneration. Accumulation of abnormal proteins through oxidative stress-induced damage, gene duplication, and/or mutation(s), oxidative stress-induced mitochondrial damage, could result in severe proteasomal dysfunction and neuronal cell death.

Prestigious Awards and Recognition

1. In 2008, Professor Halliwell was awarded the "Lifetime Achievement Award" by the Society for Free Radical Biology and Medicine in the United States for overall sustained excellence in the field.
2. 2007: Laboratory ranked number 1 worldwide by highest citation score in Free Radical Research (www .freeradicalscience.com/labs.php)
3. 2005 Research Excellence Award, School of Medicine, NUS.
4. Two research articles were recognized as Citation Classics by the ISI and a commentary published in *Current Contents.*
5. 1999, Award from ISI.
6. 2000, Chosen as a founder member of the ISI database of the world's most influential scientists in Biology and Biochemistry (see www.isihighlycited.com)
7. 2002, Awarded lifetime membership of the Oxygen Club of California in appreciation for pioneering contributions to education and research on free radicals in biological systems and their role in health and disease.
8. 2003, ranked 28 out of 5494 Biochemists/Biologists worldwide in the ISI database for the impact of publications.
9. 2003, awarded the status of "most cited scientist" in Agricultural Sciences for work on plant antioxidants, ranked 16 out of 1663 worldwide in ISI database.
10. Hirsch Index is 128.

Current Position

Professor Barry Halliwell is currently the Deputy President (in charge of research and technology) at the National University of Singapore and holds a prestigious Tan Chin Tuan Centennial Professorship in parallel. He holds appointment in the Department of Biochemistry. Professor Halliwell serves on the Editorial Boards of several journals, including *Antioxidants and Redox Signaling*, *Biochemical and Biophysical Research Communications*, *Biochemical Journal*, and *FEBS Letters*. He has been a lead speaker at Gordon Conferences and other prestigious events worldwide and is a member of several expert advisory panels to leading universities, companies, and government agencies. According to Professor Barry Halliwell, "I got into the field of free radicals and antioxidants by following up a chance observation I made during my Ph.D. work. At the time, few were interested in redox biology. Now everyone is. Don't be afraid to take a risk and explore a sudden inspiration or a new concept. You may help create a new research field and then many will follow."

I would like to end by including a tribute from a long-standing associate and collaborator of Professor Halliwell, Professor John M.C. Gutteridge: "Barry has many unique scientific qualities. Two striking examples that readily come to mind are his highly original and intellectual research creativity, and his ability to communicate science in a clear and concise way through his superb writing skills. One of my earliest recollections of collaboration with Barry was his tireless dedication each week to many hours of literature searching in the library for ever more knowledge on the topics we were researching together. Above all, however, throughout his distinguished scientific career, Barry has always been a kind, courteous, and generous mentor to the many international students privileged to be working and studying under his guidance and supervision."

Acknowledgments

Professor Halliwell wishes to acknowledge the excellent scientists and clinicians whom he has been privileged to

work with. There is insufficient space to name them all, but he would like to specially mention Professor John Gutteridge, a powerful intellect, clear thinker, and resourceful scientist, as well as Erik Anggard, Joe Bannister, David Blake, Roberto Bolli, Adrian Bomford, Dale Bredesen, John Butler, Rubens Cecchini, Christopher Chen, Marie-Veronique Clement, Carroll Cross, Victor Darley-Usmar, David Dexter, Miral Dizdaroglu, Jason Eiserich, Boris Ferger, Rudiyanto Gunawan, Andrew Holton, Robin Hoult, Duncan Hutchinson, Magnus Ingelman-Sundberg, Andrew Jenner, Peter Jenner, Kandiah Jeyaseelan, Frank Kelly, Ronan Kelly, Christiaan Leeuwenburgh, Lim Sai Kiang, John Longhurst, Sam Louie, Derek Mattey, Malcom Mitchinson, Kevin Moore, Philip Moore, Jason Morrow, Paul Motchnik, Ian Mudway, Antonia Murcia, Jaffar Nourooz-Zadeh, Choon Nam Ong, Wei Yi Ong, Bob Pasternack, Vanka Petrovic, Alain Puppo, Joseph Rafter, Abe Reznick, Catherine Rice-Evans, Raymond Seet, Kim Ping Sit, Jeremy Spencer, Benny Tan, Albert van der Vliet, Frank Watt, Markus Wenk, Thomas Westermarck, Matthew Whiteman, Helen Wiseman, Reen Wu, and Eu Leong Yong.

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Abbreviations Used

GSH = reduced glutathione
GSSG = oxidized glutathione
H_2O_2 = hydrogen peroxide
ISI = Institute of Scientific Information