Preface

All things being equal, molecules are more stable when all their electrons are paired, in electronic orbitals differing only in their spin quantum number. Therefore, free radicals — stable molecules containing unpaired electrons — have the potential to be reactive species. Their reactivity can involve taking an electron from a molecule with fully paired electrons, generating a new free radical and, potentially, initiating a chain of radical reactions. Alternatively, they can recombine with another free radical, resulting in two species with fully paired electronic orbitals. A final possibility is that they react to form a more stable species containing unpaired electrons. Predominant among these are transition metals that can stabilize unpaired electrons in their d orbitals. The chemistry of free radicals in biology and medicine is therefore interwoven with that of transition metals that are able to accept or donate electrons (e.g. iron, copper, manganese, molybdenum, vanadium, nickel and cobalt).

Free radicals were the subject of the Biochemical Society's Annual Symposium, which was held at the University of Essex, Colchester, U.K., from July 2–4 2003. The range of roles that these radical species play in biology was addressed from a variety of perspectives. Inorganic biochemists stressed the positive, and indispensable, role that protein-bound free radical species play in enzymology; cell and molecular biologists addressed our increasing awareness of the important role of free radicals in intercellular and intracellular cell signalling; and clinical perspectives were introduced via the wide range of disease processes known to be linked to uncontrolled free radical reactions. The reviews in this book are a result of presentations given at this successful interdisciplinary symposium.

Enzymology

For the reasons indicated above, the vast majority of enzymes that have evolved to produce and/or react with free radicals contain transition metal cofactors. In many cases, the inorganic redox chemistry of the transition metal is intricately linked with protein-bound organic radicals. Although the inorganic cofactors are usually protein-bound (owing to the potential biological toxicity of free iron and copper), organic cofactors can be bound (e.g. flavins) or free (e.g. quinols). The variety of the chemistry is indicated by the four chapters on enzymology. Electron transfer is fundamental to a wide range of biological catalysis. The chapter by Scrutton on flavin radicals in trimethylamine dehydrogenase explores the factors that affect electron transfer rates, the nature of protein–protein interactions between electron transferring

proteins and the role of critical amino acid residues in stabilizing protein radicals. Their studies suggest a more complex and subtle interaction between the protein electron donor and acceptor than can be explained by simple collision theory.

Protein radicals are, however, involved in more than simple electron transfer. The enzyme galactose oxidase has an active-site structure containing copper, cysteine and tyrosine, with the amino acids covalently linked by a novel thioether bond. The free radical stabilized on this amino-acid pair is required for catalysis. McPherson explore the nature of the post-translational modification that forms this enzyme's active site, concluding that the process is autocatalytic, requiring merely the presence of copper, oxygen and a N-terminal 17-amino-acid presequence (that is cleaved in the process). The detailed chemistry that drives the formation of the active site of this 'radical enzyme' is yet to be elucidated, but the plausible mechanisms all involve redox (and possible radical) chemistry.

The link between metal redox chemistry and protein free radicals has been known since the 1950s, via the work on haem peroxidases and catalases, These enzymes uses hydrogen peroxide to activate ferric (Fe^{3+}) haem iron, generating a pair of strong oxidants — protein radicals and ferryl iron ($Fe^{4+}O^{2-}$) — that can drive the oxidative chemistry required for biosynthesis (e.g. thyroid peroxidase) or host defence (e.g. neutrophil myeloperoxidase). Again, the latest work in this area is being illuminated by new structures of electron-transfer complexes. As shown by Raven, the nature of the interface between substrate (ascorbate) and enzyme (ascorbate peroxidase) suggests a far more direct and intimate association between ascorbate and the haem cofactor than was previously thought.

Nitric oxide synthase (NOS) is an enzyme that produces a free radical product, NO, via a complex five-electron oxidation of NADPH and L-citrulline. NOS has both inorganic (haem iron) and organic (tetrahydrobiopterin) cofactors. Both of these cofactors accept and donate electrons during the reaction mechanism, resulting in a plethora of free-radical and transition-metal intermediates. Indeed, the NOS mechanism can be thought of as a microcosm of the complex interplay between radical and transition metal chemistry in the body as a whole. In the article by Stuehr the novel multifunctional role of the tetrahydrobiopterin radical, in both generating the NO species on the protein and catalysing its dissociation into the cellular environment (a non-trivial process given the high affinity of NO for haem iron), is explored in detail.

Signalling

The ability of biological systems to evolve in ways that mask or accentuate the reactive chemistry of free radicals makes them ideal candidate molecules to transduce information. Although a signalling role for hydrogen peroxide (formed *in vivo* from the superoxide radical) has been in and out of vogue for some time, the 'classical' free-radical signalling molecule is NO. Again, the interplay between transition metals and free radicals is evident, as haem

enzymes are involved in the formation (see above) and transduction of the NO signal. In the latter case, as described by Koesling, it is the binding of NO to the ferrous haem enzyme guanylate cyclase that activates the formation of the second messenger (cGMP). Detailed three-dimensional mapping of the NO-producing and -responding cells is now possible in the intact insect brain, as discussed by Trimmer.

As indicated above, oxygen-radical signalling has taken a back seat to that of nitrogen radicals in recent years. This is about to change with the discovery of a ubiquitous class of enzymes that make superoxide and hydrogen peroxide, Nox and Duox respectively, from molecular oxygen and NADPH. While previously limited to the 'bacteria-killing' phagocyte oxidase, the range of functions of Nox/Duox enzymes, as described by Lambeth, is now linked to variety of signalling processes, e.g. mitogenic growth, apoptosis and angiogenesis. In the case of apoptosis, Burkitt argues that the release of the mitochondrial haem protein cytochrome *c* into the cytoplasm may intersect with peroxide signalling pathways and result in the uncontrolled production of ferryl haem and protein radicals. Unlike the peroxidases described by Lambeth and Raven, the protein structure of cytochrome *c* does not lend itself to controlling the reactivity of these oxidants and, therefore, undesirable free radical oxidative chemistry can occur.

Just as we learn more about the range of enzyme systems that can produce free radicals, so we are learning about new potential targets. Trimmer and Darley-Usmar argue that other haem proteins (in particular, the major oxygen-consuming enzyme in aerobic organisms, mitochondrial cytochrome *c* oxidase) are controlled by NO inhibition, and that this inhibition plays a role in signalling pathways, e.g. in controlling the flashing response in fireflies. Redox signalling has long been thought of as synonymous with the reduction or oxidation of protein thiols. However, as discussed in detail by Darley-Usmar, S-nitrosation of thiols may also be important. Indeed, he argues that there is likely to be a complex interaction between oxygen and nitrogen radical signalling pathways throughout the cell.

Disease

Notwithstanding their importance in enzymology and signalling, the uncontrolled formation of free radicals (and associated reactive oxygen and nitrogen species) plays a major role in the pathophysiology of a wide range of diseases. Münzel suggests that the increase in the levels of radical species induced by diabetes, smoking, hypertension and increases in cholesterol can induce vascular dysfunction with associated cardiovascular risk. Patel and Mann address the specific case of overproduction of NO. Patel argues that in the cases of sepsis, this allows NO to reach anomalous levels in the erythrocyte, forming S-nitrosated haemoglobin and mediating the chronic hypotension observed clinically. Mann argues that pregnancy-associated diseases (e.g. gestational diabetes, intrauterine growth retardation and pre-eclampsia) affect NO signalling pathways, altering endothelial-cell function in such a way as to have significant implications for the fetal cardiovascular system.

Organisms adapt in a range of ways to this radical onslaught; both 'prevention' and 'cure' are employed. Antioxidants can 'mop up' reactive species (e.g. vitamins C and E reduce free radicals by donating electrons, forming stable radical species, and hence can terminate radical chain reactions). Mathers addresses the response to redox stress at the whole organism level in model systems — *Caenorhabditis elegans* and *Drosophila melonogaster* — using microarray and proteomics approaches to map the genes that are activated and the proteins that are synthesized. Motterrlini focuses on the unappreciated antioxidant properties of the bile pigments biliverdin and bilirubin, arguing strongly that, rather than being mere haem-breakdown products, their formation by the inducible isoform of the enzyme haem oxygenase is a major antioxidant defence system in the body.

Finally a persuasive case is made that two proteins with very different 'headline' functions are, in fact, involved in preventing radical formation *in vivo*. Brown states that the copper-binding function of the prion protein points to its major 'normal' biological function by being that of an enzyme that removes superoxide (by analogy with the more established Cu/Zn superoxide dismutase). It is then argued that it is the loss of copper binding (and the related antioxidant activity) in the aberrant prion proteins that results in diseases such as scrapie and Creutzfeldt–Jakob disease.

The 'classical' function of mitochondrial uncoupling proteins, present in the mitochondria of brown fat, is heat production [the uncoupling protein 1 (UCP1) isoform decreasing the mitochondrial membrane potential to a level that allows rapid oxygen consumption with minimal ATP production, with the resulting inefficiency inducing maximal heat production]. Brand promotes a completely different role for the more widely expressed UCP isoforms (UCP2 and UCP3) based on the fact that superoxide formation in mitochondria only occurs at high membrane potentials. The mechanism proposed is that activation of the UCPs leads to mild uncoupling, thereby decreasing the mitochondrial membrane potential to the level where superoxide production stops. Activation appears to require specific lipid oxidation products (hydroxynonenals) formed via superoxide-induced lipid peroxidation. Here, the reactive species itself has a signalling role, ultimately bringing about its own destruction.

One book cannot hope to cover the wide variety of reactions catalysed by free radicals, their multifaceted role in signalling, or the range of diseases in which they are implicated. However, by giving a flavour of these different areas it has been our intention to indicate where they overlap and thus illustrate possible future research directions.

Chris Cooper, Mike Wilson
and Victor Darley-Usmar

Abbreviations

ACE	angiotensin-converting enzyme
ACh	acetylcholine
APX	ascorbate peroxidase
ARE	antioxidant-responsive element
ASK	apoptosis signal-regulating kinase
BH_4	tetrahydrobiopterin
BVR	biliverdin reductase
bZIP	basic leucine zipper
$[Ca^{2+}]_i$	intracellular calcium concentration
CaM	calmodulin
CAT	cationic amino acid transporter
C*c*P	cytochrome *c* peroxidase
CGD	chronic granulomatous disease
CNC	cap 'n' collar
CNS	central nervous system
COX	cytochrome *c* oxidase
cyt *c*	cytochrome *c*
DAF-2	4,5-diaminofluorescein
DAF-2-DA	4,5-diaminofluorescein diacetate
DCF	2′,7′-dichlorofluorescein
$DCFH_2$	2′,7′-dichlorofluorescin
DEA-NO	diethylamine NONOate
DEM	diethyl maleate
DMPO	5,5-dimethyl-1-pyrroline *N*-oxide
DMPOX	5,5-dimethyl-1-pyrrolidone-2-oxyl
2D-PAGE	two-dimensional PAGE
DPI	diphenylene iodinium
eNOS	endothelial nitric oxide synthase
ETF	electron-transferring flavoprotein
Fc^+	ferricenium
GC	guanylate (quanlyl) cyclase
GCL	glutamate cysteine ligase
GO	galactose oxidase
G6PDH	glucose-6-phosphate dehydrogenase
GST	glutathione S-transferase
H_4B	6*R*-tetrahydrobiopterin
Hb	haemoglobin
HO-1, -2, -3	haem oxygenases 1, 2 and 3
HR	hypersensitive response

HRP	horseradish peroxidase
hsp/Hsp	heat-shock protein
iNOS	inducible nitric oxide synthase
JNK	c-Jun N-terminal protein kinase
LDL	low-density lipoprotein
mAChR	muscarinic acetylcholine receptor
MAPK	mitogen-activated protein kinase
MEK	mitogen-activated protein kinase/ extracellular-signal-regulated kinase kinase
metHb	methaemoglobin
mitoPBN	phenylbutylnitrone covalently linked to triphenylphosphonium
mitoQ	ubiquinone covalently linked to triphenylphosphonium
MNP	2-methyl-2-nitrosopropane
nAChR	nicotinic acetylcholine receptor
L-NAME	N^G-nitro-L-arginine methyl ester
NF-E2	nuclear factor-erythroid 2
NF-κB	nuclear factor κB
NMDA	*N*-methyl-D-aspartate
NOHA	N-hydroxy-L-arginine
NOS	nitric oxide synthase
NQO	NAD(P)H:quinone oxidoreductase
Nrf	nuclear factor-erythroid 2 p45-related factor
ODQ	1H-[1,2,4]oxadiazolo[4,3-a]-quinoxalin-1-one
oxyHb	oxyhaemoglobin
$p42/p44^{MAPK}$	p42/p44 mitogen-activated protein kinase
PBN	phenylbutylnitrone
PERK	protein kinase R-like endoplasmic reticulum factor
PI 3-kinase	phosphoinositide 3-kinase
PKB	protein kinase B
PKC	protein kinase C
PPAR-γ	peroxisome proliferator-activated receptor-γ
PrP^c	cellular form of the prion protein
PrP^{Sc}	abnormal form of the prion protein
Prx	peroxiredoxin
PSD-95	post-synaptic density protein-95
rboh	respiratory burst oxidase homologue
RNAi	RNA interference
RNS	reactive nitrogen species
ROS	reactive oxygen species
rpAPX	recombinant pea cytosolic ascorbate peroxidase
rsAPX	recombinant soya bean cytosolic ascorbate peroxidase
sGC	soluble guanylate cyclase
SKN-1	skinhead-1
SNOHb	*S*-nitrosohaemoglobin
SOD	superoxide dismutase
tBHQ	t-butylhydroquinone
TMADH	trimethylamine dehydrogenase

UAS	upstream activating sequence
UCP	uncoupling protein
VEGF	vascular endothelial growth factor
VNL	lateral branch of the ventral nerve
YC-1	3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole