

Contents

Preface — v

List of contributing authors — xix

Patricia C. Dos Santos and Dennis R. Dean

1 A retrospective on the discovery of [Fe-S] cluster biosynthetic machineries in *Azotobacter vinelandii* — 1

- 1.1 Introduction — 1
- 1.2 An introduction to nitrogenase — 3
- 1.3 Approaches to identify gene-product and product-function relationships — 7
- 1.4 FeMoco and development of the scaffold hypothesis for complex [Fe-S] cluster formation — 7
- 1.5 An approach for the analysis of *nif* gene product function — 10
- 1.5.1 Phenotypes associated with loss of *NifS* or *NifU* function indicate their involvement in nitrogenase-associated [Fe-S] cluster formation — 11
- 1.5.2 *NifS* is a cysteine desulfurase — 12
- 1.5.3 Extension of the scaffold hypothesis to *NifU* function — 16
- 1.5.4 Discovery of *isc* system for [Fe-S] cluster formation and functional cross-talk among [Fe-S] cluster biosynthetic systems — 22
- 1.6 The *isc* system is essential in *A. vinelandii* — 24
- 1.7 There is limited functional cross-talk between the *Nif* and *isc* systems — 25
- 1.8 Closing remarks — 26
- Acknowledgments — 26
- References — 26

Laurent Aussel, Sylvia Chareyre, Yohann Duverger, Benjamin Ezraty, Allison Huguenot, Pierre Mandin, Béatrice Py, Jordi Zamarreno, and Frédéric Barras

2 The ISC system and the different facets of Fe-S biology in bacteria — 31

- 2.1 Introduction — 31
- 2.2 The ISC system, the general housekeeping system for Fe-S biogenesis — 31
- 2.2.1 Description and function — 31
- 2.2.2 The putative role of Fxn in early Fe-S biogenesis by the ISC system — 34
- 2.2.3 Stress represses ISC functions and enhances SUF pathway activity — 34
- 2.3 Genetic regulation of ISC synthesis — 35
- 2.4 The role of the ISC system in antibiotic resistance — 36

- 2.4.1 The proton motive force link — 36
- 2.4.2 The DNA repair connection — 38
- 2.5 The role of the ISC system in bacterial pathogenesis — 38
- 2.6 Conclusions — 40
- References — 41

F. Wayne Outten

- 3 **A stress-responsive Fe-S cluster biogenesis system in bacteria – the *suf* operon of Gammaproteobacteria — 47**
 - 3.1 Introduction to Fe-S cluster biogenesis — 47
 - 3.2 Sulfur trafficking for Fe-S cluster biogenesis — 48
 - 3.3 Iron donation for Fe-S cluster biogenesis — 49
 - 3.4 Fe-S cluster assembly and trafficking — 51
 - 3.5 Iron and oxidative stress are intimately intertwined — 53
 - 3.6 Stress-response Fe-S cluster biogenesis in *E. coli* — 56
 - 3.7 Sulfur trafficking in the stress-response Suf pathway — 57
 - 3.8 Stress-responsive iron donation for the Suf pathway — 61
 - 3.8.1 SufD — 61
 - 3.8.2 Iron storage proteins — 63
 - 3.8.3 Other candidates — 64
 - 3.9 Unanswered questions about Suf and Isc roles in *E. coli* — 65
 - Acknowledgment — 65
 - References — 66

Erin L. Mettert, Nicole T. Perna, and Patricia J. Kiley

- 4 **Sensing the cellular Fe-S cluster demand: a structural, functional, and phylogenetic overview of *Escherichia coli* IscR — 75**
 - 4.1 Introduction — 75
 - 4.2 General properties of IscR — 76
 - 4.3 [2Fe-2S]-IscR represses Isc expression *via* a negative feedback loop — 78
 - 4.4 IscR adjusts synthesis of the Isc pathway based on the cellular Fe-S demand — 80
 - 4.5 IscR has a global role in maintaining Fe-S homeostasis — 82
 - 4.6 Fe-S cluster ligation broadens DNA site specificity for IscR — 83
 - 4.7 Phylogenetic analysis of IscR — 85
 - 4.8 Binding to two classes of DNA sites allows IscR to differentially regulate transcription in response to O₂ — 89
 - 4.9 Roles of IscR beyond Fe-S homeostasis — 91
 - 4.10 Additional aspects of IscR regulation — 91
 - 4.11 Summary — 92
 - Acknowledgments — 92
 - References — 92

Patricia C. Dos Santos

5	Fe-S assembly in Gram-positive bacteria — 97
5.1	Introduction — 97
5.2	Fe-S proteins in Gram-positive bacteria — 97
5.3	Fe-S cluster assembly orthologous proteins — 99
5.3.1	Clostridia-ISC system — 99
5.3.2	Actinobacteria-SUF — 104
5.3.3	Bacilli-SUF — 105
5.4	Concluding remarks and remaining questions — 112
	References — 113

Debkumar Pain and Andrew Dancis

6	Fe-S cluster assembly and regulation in yeast — 117
6.1	Introduction — 117
6.2	Yeast and Fe-S cluster assembly – evolutionary considerations — 117
6.2.1	Nfs1 and the surprise of Isd11 — 118
6.2.2	Scaffold proteins in yeast mitochondria — 119
6.2.3	Frataxin's roles throughout evolution — 120
6.2.4	Ssq1 is a specialized Hsp70 chaperone arising by convergent evolution — 121
6.2.5	Atm1 and CIA components — 121
6.2.6	Yeast components are conserved with their human counterparts — 122
6.2.7	Yeast Fe-S cluster assembly mutants modeling aspects of human diseases — 123
6.3	Yeast genetic screens pointing to the Fe-S cluster assembly apparatus — 124
6.3.1	Misregulation of iron uptake — 124
6.3.2	Suppression of <i>Δsod1</i> amino acid auxotrophies — 125
6.3.3	tRNA modification and the <i>SPL1-1</i> allele — 126
6.3.4	tRNA thiolation and resistance to killer toxin — 126
6.3.5	Cytoplasmic aconitase maturation — 126
6.3.6	Ribosome assembly — 127
6.3.7	Synthetic lethality with the <i>pol3-13</i> allele — 127
6.3.8	Factors needed for Yap5 response to high iron — 128
6.3.9	Screening of essential genes coding for mitochondrial proteins — 129
6.4	Mitochondrial Fe-S cluster assembly — 129
6.4.1	Mitochondrial cysteine desulfurase — 131
6.4.2	Formation of the Isu Fe-S cluster intermediate in mitochondria — 135
6.4.3	Roles of frataxin — 136
6.4.4	Bypass mutation in Isu — 137
6.4.5	Transfer of the mitochondrial Isu Fe-S cluster intermediate — 138
6.4.6	Role of Grx5 — 138

6.4.7	The switch between cluster synthesis and cluster transfer — 139
6.5	Role of glutathione — 140
6.5.1	Glutathione and monothiol glutaredoxins in mitochondria — 141
6.5.2	Glutathione and monothiol glutaredoxins Grx3 and Grx4 outside of mitochondria — 142
6.6	Role of Atm1, an ABC transporter of the mitochondrial inner membrane — 143
6.6.1	Cells lacking Atm1 lose mtDNA — 144
6.7	Relationship between Fe-S cluster biogenesis and iron homeostasis — 146
6.8	Conclusion and missing pieces — 152
	Acknowledgments — 153
	References — 153

Caryn E. Outten

7	The role of Fe-S clusters in regulation of yeast iron homeostasis — 161
7.1	Introduction — 161
7.2	Iron acquisition and trafficking in yeast — 161
7.3	Regulation of iron homeostasis in <i>S. cerevisiae</i> — 164
7.3.1	Aft1/Aft2 low-iron transcriptional regulators and target genes — 164
7.3.2	Yap5 high-iron transcriptional regulator and target genes — 166
7.3.3	Links between mitochondrial Fe-S cluster biogenesis, the Grx3/Grx4/Fra2/Fra1 signaling pathway, and Aft1/Aft2 regulation — 167
7.3.4	Fe-S cluster binding by Grx3/4 and Fra2 is important for their function in <i>S. cerevisiae</i> iron regulation — 168
7.3.5	Working model for Fe-dependent regulation of Aft1/2 via the Fra1/Fra2/Grx3/Grx4 signaling pathway — 170
7.3.6	Yap5 regulation and mitochondrial Fe-S cluster biogenesis — 172
7.4	Regulation of iron homeostasis in <i>S. pombe</i> — 173
7.4.1	Fep1 and Php4 transcriptional repressors and target genes — 173
7.4.2	Roles for Grx4 in regulation of Fep1 and Php4 activity — 176
7.4.3	Molecular basis of iron-dependent control of Fep1 activity — 178
7.4.4	Molecular basis of iron-dependent control of Php4 activity — 179
7.5	Summary — 180
	Acknowledgments — 181
	References — 181

Tracey Rouault

8	Biogenesis of Fe-S proteins in mammals — 187
8.1	Introduction — 187
8.2	The Fe-S regulatory switch of IRP1 — 187

- 8.3 IRP2, a highly homologous gene, also posttranscriptionally regulates iron metabolism, but iron sensing occurs through regulation of its degradation rather than through a Fe-S switch mechanism — 191
- 8.4 Identification of the mammalian cysteine desulfurase and two scaffold proteins: implications for compartmentalization of the process — 192
- 8.5 Sequential steps in Fe-S biogenesis – an initial Fe-S assembly process on a scaffold, followed by Fe-S transfer to recipient proteins, aided by a chaperone-cochaperone system — 193
- 8.6 Mitochondrial iron overload in response to defects in Fe-S biogenesis raises important questions about how mitochondrial iron homeostasis is regulated — 196
- 8.7 Perspectives and future directions — 197
- References — 198

Nunziata Maio and Tracey A. Rouault

- 9 **Delivery of iron-sulfur clusters to recipient proteins: the role of chaperone and cochaperone proteins — 205**
 - 9.1 Introduction — 205
 - 9.2 A specialized chaperone-cochaperone system ensures efficient Fe-S cluster delivery — 205
 - 9.3 Transfer of Fe-S clusters to recipient proteins: the ATPase cycle — 210
 - 9.4 The mammalian Fe-S transfer system — 212
 - 9.5 Recent progress: identification of molecular features that guide selection of recipient Fe-S proteins by the Fe-S transfer complex — 215
 - 9.6 SDHAF1, a member of the LYR motif family, assists Fe-S cluster incorporation into SDHB — 218
 - 9.7 Potential role of LYR motif proteins in Fe-S cluster biogenesis — 218
 - 9.8 Molecular features of peptides containing the LYR motif that affect binding to HSC20 — 220
 - 9.9 Conclusions and future perspectives — 220
 - References — 221

Wing Hang Tong

- 10 **Iron-sulfur proteins and human diseases — 227**
 - 10.1 Introduction — 227
 - 10.2 Oxidative susceptibility of Fe-S proteins — 228

10.2.1	Aconitases: targets of oxidative stress in disease and aging — 230
10.3	Diseases associated with genetic defects in Fe-S proteins — 234
10.3.1	Mitochondrial respiratory complexes and human diseases — 234
10.3.2	FECH deficiency causes erythropoietic protoporhyria (MIM 177000) — 238
10.3.3	DNA repair Fe-S proteins and human disorders — 239
10.3.4	Diseases associated with genetic defects in radical S-adenosylmethionine enzymes — 242
10.3.5	DNA polymerase delta 1 (POLD1) in mandibular hypoplasia, deafness, progeroid features, and lipodystrophy (MDPL) and cancer — 245
10.3.6	CDGSH iron sulfur domain (CISD) proteins — 245
10.4	Diseases associated with genetic defects in Fe-S cluster biogenesis — 248
10.4.1	A GAA trinucleotide repeat expansion in FXN is the major cause of the neurodegenerative disorder Friedreich ataxia — 250
10.4.2	Mutations in ABCB7 cause x-linked sideroblastic anemia with ataxia — 255
10.4.3	Mutations in GLRX 5 cause an autosomal recessive pyridoxine-refractory sideroblastic anemia — 256
10.4.4	Mutations in ISCU cause myopathy with lactic acidosis (MIM 255125) — 258
10.4.5	NUBPL mutations cause childhood-onset mitochondrial encephalomyopathy and respiratory complex I deficiency (MIM252010) — 261
10.4.6	Mutations in NFU1 cause multiple mitochondrial dysfunctions syndrome 1 (MIM 605711) — 262
10.4.7	Mutations in BOLA3 cause MMDS 2 (MIM 614299) — 264
10.4.8	IBA57 deficiency causes severe myopathy and encephalopathy — 265
10.4.9	A mutation in ISD11 causes deficiencies of respiratory complexes — 266
10.4.10	Infantile mitochondrial complex II/III deficiency (IMC23D) caused by a missense mutation in NFS1 — 267
10.4.11	Mutations in HSPA9 in patients with congenital sideroblastic anemia and myelodysplastic syndrome — 268
10.5	Fe-S cluster biogenesis and iron homeostasis — 269
10.6	Therapeutic strategies — 270
	Acknowledgments — 272
	References — 272

Simon A.B. Knight and Robert B. Wilson

- II Friedrich ataxia — 307**
 - II.1 Introduction — 307
 - II.2 Clinical presentation and genetics — 308
 - II.2.1 Signs and symptoms — 308
 - II.2.2 Identification of the disease gene — 308
 - II.2.3 Mitochondrial dysfunction — 309
 - II.3 Iron metabolism and dysregulation — 309
 - II.3.1 Mitochondrial iron accumulation — 309
 - II.3.2 Oxidative stress — 310
 - II.3.3 ISC biogenesis — 310
 - II.3.4 Precise function of frataxin — 311
 - II.3.5 Cellular consequences of frataxin deficiency — 312
 - II.4 Summary — 313
 - References — 314

Silke Leimkühler

- 12 Connecting the biosynthesis of the molybdenum cofactor, Fe-S clusters, and tRNA thiolation in humans — 319**
 - 12.1 Introduction — 319
 - 12.2 Pathways for the formation of Moco and thiolated tRNAs in humans — 321
 - 12.2.1 Moco biosynthesis in mammals — 321
 - 12.2.2 The role of tRNA thiolation in the cell — 331
 - 12.3 The connection between sulfur-containing biomolecules and their distribution in different compartments in the cell — 333
 - 12.3.1 Sulfur transfer in mitochondria — 333
 - 12.3.2 Sulfur transfer in the cytosol — 335
 - 12.3.3 Role of NFS1, ISD11, URM1, and MOCS2A in the nucleus — 338
 - Acknowledgments — 340
 - References — 340

Kerstin Gari

- 13 Iron-sulphur proteins and genome stability — 347**
 - 13.1 The importance of genome stability — 347
 - 13.2 Link between iron-sulphur cluster biogenesis and genome stability — 348
 - 13.3 FeS proteins in DNA replication — 350
 - 13.3.1 DNA primase and DNA polymerase alpha — 351
 - 13.3.2 DNA polymerases delta and epsilon — 352
 - 13.3.3 DNA2 — 353
 - 13.4 FeS proteins in DNA repair — 354
 - 13.4.1 DNA glycosylases — 355

- 13.4.2 The Rad3 family of helicases — 357
- 13.5 Outlook — 361
- References — 361

Roland Lill, Marta A. Uzarska and James Wohlschlegel

14 Eukaryotic iron-sulfur protein biogenesis and its role in maintaining genomic integrity — 369

- 14.1 Introduction — 369
- 14.2 Biogenesis of mitochondrial Fe-S proteins — 374
- 14.2.1 Step 1: *De novo* Fe-S cluster assembly on the Isu1 scaffold protein — 374
- 14.2.2 Step 2: Chaperone-dependent release of the Isu1-bound Fe-S cluster — 375
- 14.2.3 Step 3: Late-acting ISC assembly proteins function in [4Fe-4S] cluster synthesis and in target-specific Fe-S cluster insertion — 377
- 14.3 The role of the mitochondrial ABC transporter Atm1 in the biogenesis of cytosolic and nuclear Fe-S proteins and in iron regulation — 380
- 14.4 The role of the CIA machinery in the biogenesis of cytosolic and nuclear Fe-S proteins — 382
- 14.4.1 Step 1: The synthesis of a [4Fe-4S] on the scaffold complex Cfd1-Nbp35 — 382
- 14.4.2 Step 2: Transfer of the [4Fe-4S] cluster to target apo-proteins — 382
- 14.5 Specialized functions of the human CIA-targeting complex components — 383
- 14.5.1 Dedicated biogenesis of cytosolic and nuclear Fe-S proteins — 383
- 14.5.2 The dual role of CIA2A in iron homeostasis — 384
- 14.6 Fe-S protein assembly and the maintenance of genomic stability — 385
- 14.6.1 Late-acting CIA factors in DNA metabolism — 386
- 14.6.2 XPD and the Rad3 family of DNA helicases — 387
- 14.6.3 Fe-S proteins involved in DNA replication — 388
- 14.6.4 DNA glycosylases as Fe-S proteins — 389
- 14.7 Biochemical functions of Fe-S clusters in DNA metabolic enzymes — 389
- 14.8 Interplay among Fe-S proteins, genome stability, and tumorigenesis — 391
- 14.9 Summary — 393
- Acknowledgments — 394
- References — 394

Phillip L. Bartels, Elizabeth O'Brien, and Jacqueline K. Barton

- 15 **DNA signaling by iron-sulfur cluster proteins — 405**
- 15.1 Introduction — 405
- 15.2 DNA-mediated signaling in BER — 405
- 15.3 Assessing redox signaling by [4Fe-4S] proteins *in vitro* and *in vivo* — 411
- 15.4 DNA CT in other repair pathways — 415
- 15.5 A role for CT in eukaryotic DNA replication? — 417
- 15.6 DNA-binding [4Fe-4S] proteins in human disease — 419
- 15.7 Conclusions — 420
- Acknowledgments — 420
- References — 421

Hong Ye

- 16 **Iron-sulfur cluster assembly in plants — 425**
- 16.1 Introduction — 425
- 16.2 Iron uptake, translocation, and distribution — 425
- 16.3 Fe-S cluster assembly — 427
- 16.3.1 SUF system in plastids — 429
- 16.3.2 ISC system in mitochondria — 432
- 16.3.3 CIA system in cytosol — 434
- 16.4 Regulation of cellular iron homeostasis by Fe-S cluster biosynthesis — 436
- 16.5 Conservation of Fe-S cluster assembly genes across the green lineage — 436
- 16.6 Potential significance to agriculture — 438
- Acknowledgments — 439
- References — 439

Eric S. Boyd, Gerrit J. Schut, Eric M. Shepard, Joan B. Broderick,
Michael W. W. Adams and John W. Peters

- 17 **Origin and evolution of Fe-S proteins and enzymes — 445**
- 17.1 Introduction — 445
- 17.2 Fe-S chemistry and the origin of life — 445
- 17.3 The ubiquity and antiquity of biological Fe-S clusters — 448
- 17.4 Early energy conversion — 452
- 17.5 Evolution of complex Fe-S cluster containing proteins — 456
- 17.6 The path from minerals to Fe-S proteins and enzymes — 458
- References — 459

Index — 463