Subsystem: Fe-S cluster assembly

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Iron-sulfur (Fe-S) proteins are present in all living organisms and play important roles in electron transport and metalloenzyme catalysis [1]. Although Fe-S clusters can be assembled into proteins *in vitro* from Fe^{2+} and S_2, it is clear that *in vivo* this process must be facilitated by protein factors to avoid accumulation of Fe^{2+} and S_2 to toxic levels. The complex multi-step process involved in the biosynthesis of Fe-S clusters is only now being clarified, and early work has identified three distinct systems termed NIF (nitrogen fixation), ISC (iron-sulfur cluster), and SUF (sulfur mobilization).

NifS-NifU are required for the assembly of Fe-S clusters of the nitrogenase proteins in *Azotobacter vinelandii* [2]. NifS is a pyridoxal phosphate-dependent cysteine desulfurase that initiates Fe-S cluster formation by eliminating elemental sulfur from cysteine and transferring it to NifU, which serves as a scaffold for the assembly of Fe-S clusters prior to their delivery to apo–Fe-S protein targets.

In contrast to the NIF machinery that specifically deals with the maturation of nitrogenase, the ISC proteins are involved in the general biosynthesis pathway for numerous Fe-S proteins [3]. In several bacteria the genes encoding these Fe-S assembly proteins (IscS, IscU, IscA, Hsc66, Hsc20, and ferredoxin) are organized in a cluster *iscSUA-hscBA-fdx*. Mutation of these genes in *Escherichia coli* decreases the activity of many Fe-S proteins, whereas overexpression of the operon leads to increased production of Fe-S proteins [4].

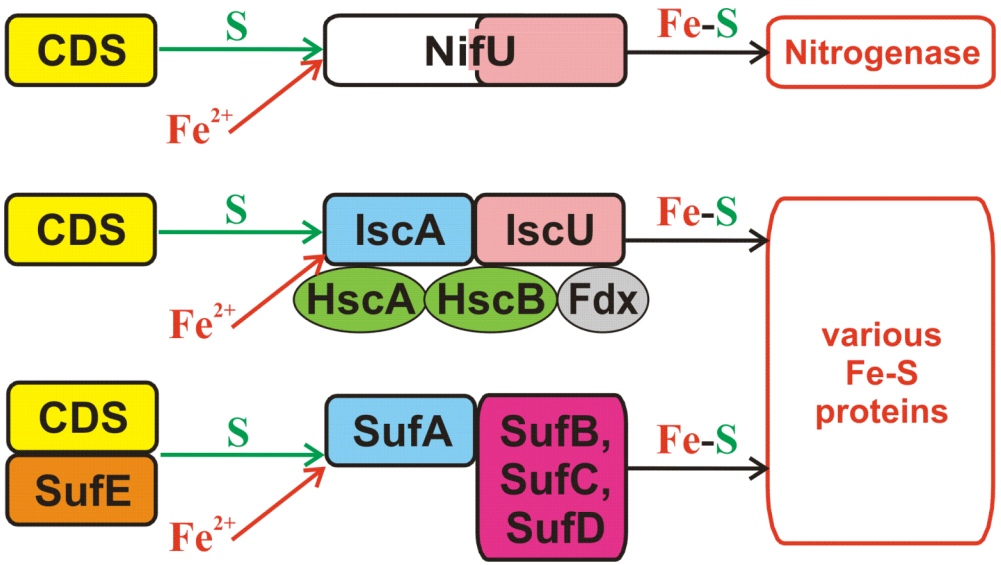
A third system (SUF) encoded by the *sufABCDSE* operon represents a minor pathway for the assembly of Fe-S clusters [5]. In an *E. coli* mutant, from which the entire *isc* operon was deleted, the activity of Fe-S proteins was only 2–10% that in wild-type cells. The residual activity may arise from the contribution of the SUF system, since overexpression of the *suf* operon restores the growth phenotype and activity of Fe-S proteins in mutant cells lacking the ISC machinery. Disruption of the *suf* operon does not cause any major defects, whereas the loss of both the ISC and SUF systems leads to synthetic lethality.

Several similarities have been found among the NIF, ISC, and SUF systems. IscS and SufS are structurally similar to NifS, and all three function as a cysteine desulfurase [6]. IscU is homologous to the N-terminal domain of NifU and contains three conserved cysteine residues that are essential for its function as a scaffold for intermediate Fe-S clusters [7]. SufA and IscA are members of the HesB protein family that binds iron and [2Fe-2S] clusters, and mediates iron delivery for assembly of Fe-S clusters in IscU [8]. The ISC machinery contains three additional components, two essential chaperones HscB and HscA, and nonessential [2Fe-2S]ferredoxin Fdx. The biochemical properties of the SUF-specific components are less well understood. SufE interacts with SufS and stimulates its cysteine desulfurase activity [9]. The SufB, SufC, and SufD proteins associate in a stable complex, and SufC has been shown to possess ATPase activity in this context [10].

Mutational analysis has demonstrated that the ISC system predominantly functions in the biosynthesis of Fe-S proteins, whereas the SUF system contributes only modestly [5]. The expression of the *suf* operon is controlled by OxyR and Fur, suggesting a stress response function for the SUF machinery under conditions of oxidative stress (when FeS clusters are damaged) and iron limitation [11, 12].

Recent functional complementation study of the *E. coli isc-suf* double mutant showed that *nifSU*-like genes cloned from *Helicobacter pylori* are functionally exchangeable with the *isc* and *suf* operons under anaerobic conditions [13]. Thus, at least some NIF-like systems participates in the maturation of a wide variety of Fe-S proteins. However, the NIF system has only been found in a limited number of bacterial species, mostly anaerobes.

Fig. 1. Fe-S cluster assembly. Subsystem diagram.



#	Abbrev.	Functional Role
1	CDS	Cysteine desulfurase (EC 4.4.1.-)
2	IscA	Iron binding protein IscA for iron-sulfur cluster assembly
3	SufA	Iron binding protein SufA for iron-sulfur cluster assembly
4	SufA2	probable iron binding protein for iron-sulfur cluster assembly
5	HesB	probable iron binding protein from the HesB_IscA_SufA family
6	IscU	Iron-sulfur cluster assembly scaffold protein IscU
7	NifU	Iron-sulfur cluster assembly scaffold protein NifU
8	SufE	Sulfur acceptor protein SufE for iron-sulfur cluster assembly
9	SufD	Iron-sulfur cluster assembly protein SufD
10	SufB	Iron-sulfur cluster assembly protein SufB
11	SufC	Iron-sulfur cluster assembly ATPase protein SufC
12	HscB	Chaperone protein hscB
13	HscA	Chaperone protein hscA
14	Fdx	Ferredoxin, 2Fe-2S
15	IscR	Iron-sulfur cluster regulator IscR
16	SufR	Iron-sulfur cluster regulator SufR

Components from the same protein family:

IscS, SufS, NifS

IscA, SufA,HesB

IscU, NifU

SufB, SufD

Similar to ABC transporters ATPases

Similar to DnaK-DnaJ molecular chaperones

Fig. 2. Fe-S cluster assembly . Subsystem spreadsheet.

Organism	Variant Code	Sulfur transfer: Cysteine desulfurase (CDS)	Iron-binding: IscA-2, SufA-3, SufA2-4, HesB-5	Scaffold: IscU-6, NifU-7	SufE	SufD	SufB	SufC	Chaperones : HscB-12, HscA-13	Fdx
Escherichia coli K12	1	1664, 2500, 2766	2498-2, 1668-3, 157-5	2499-6	1663, 2767	1665	1667	1666	2497-12, 2496-13	2495
Chlamydophila pneumoniae AR39	10	354, 56			693	55	53	54		
Pyrococcus furiosus	100	1103, 167				1329	1330	1331		
Methanocaldococcus jannaschii	1000						34	35		
Thermoplasma volcanium	2000		1393-4			1389	1390	1391		
Mycobacterium tuberculosis CDC1551	11	1552, 3216	1554-4, 2323-5	1553-6	3503	1550	1549	1551		
Erwinia carotovora	111	2606, 3010, 3844, 3965	2604-2, 3969-3, 4492-5	2605-6, 3845-7	3011, 3964	3966	3968	3967	2603-12, 2602-13	2601
Prochlorococcus marinus str. MIT 9313	2	1597, 2037, 716	1961-5		1140	1598	1600	1599		
Burkholderia pseudomallei K96243	3	4159, 4250, 6551, 782	4252-2, 4158-4, 3382-5	4251-6, 6621-6		4160	4162	4161	4253-12, 4254-13	4255
Pirellula sp.	33	1940, 4145, 5102, 5876	2543-5	1939-6		5520	5521	5522		
Haemophilus influenzae Rd KW20	4	1234, 1278, 352	350-2, 1635-5	351-6	1233				349-12, 347-13	346
Azotobacter vinelandii	40	1435, 2228, 3056, 4190, 548	1433-2, 2230-2, 313-5	1434-6, 2229-7	1320				1432-12, 1431-13	1430
Anopheles gambiae [E]	44	11207, 13036	12371-5, 4948-5	1256-6	13037					2254
Neisseria meningitidis Z2491	5	1453	1456-2, 681-5	1455-6					1457-12, 1230-13	1233
Aquifex aeolicus VF5	55	523, 730	1292-5	622-6						
Leuconostoc mesenteroides	6	1009		1010-6		1008	1011	1007		
Chlorobium tepidum TLS	66	1965		1964-6						
Campylobacter jejuni RM1221	7	284		283-7						
Geobacter sulfurreducens PCA	77	1855, 1998, 2554, 2768		1399-6, 1999-7						
Bacillus subtilis	8	267, 2754, 2791, 2962, 3274	3222-5	3273-6		3275	3272	3276		
Nostoc sp. PCC 7120	9	1766, 2802, 2812, 3395, 3517, 4174	1741-5, 2692-5, 4648-5	1765-7	3820	2801	2799	2800		

Functional variants:

#1: complete ISC and SUF systems;
#111: complete ISC, SUF, NIF systems;
#10: SUF: SufBCD+SufE+SufS;
#100: SUF: SufBCD+SufS;
#1000: SUF: only SufBC;
#2000: SUF: only SufABC;
#2: ISC: only IscA+IscS, SUF - complete;
#3: ISC: complete, SUF lacks SufE;
#33: ISC: IscA+IscU+IscS, SUF lacks SufE;
#4: ISC: complete, SUF: only SufE;
#40: ISC: complete, SUF: only SufE, NIF
#44: ISC: IscA+IscU+IscS, SUF: only SufE;
#8: ISC: IscA+IscU+IscS, SUF: SufBCD;
#5: ISC - complete;
#55: ISC: IscA+IscU+IscS;
#6: ISC: only IscS+IscU, SUF: SufBCD;
#66: ISC: only IscS+IscU;
#7: NIF: NifU+NifS;
#77: NIF: NifU+NifS; ISC: IscS+IscU
#9: NIF: IscA+NifU+NifS, SUF: SufBCD+SufE.

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