UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO SCIENZE DELLA VITA E DELL'AMBIENTE

Corso di Laurea

Scienze Biologiche

TITOLO TESI (ITALIANO)

Ripiegamento non corretto indotto dall'ossidazione e aggregazione della Superossido Dismutasi, implicazioni per la Sclerosi Laterale Amiotrofica.

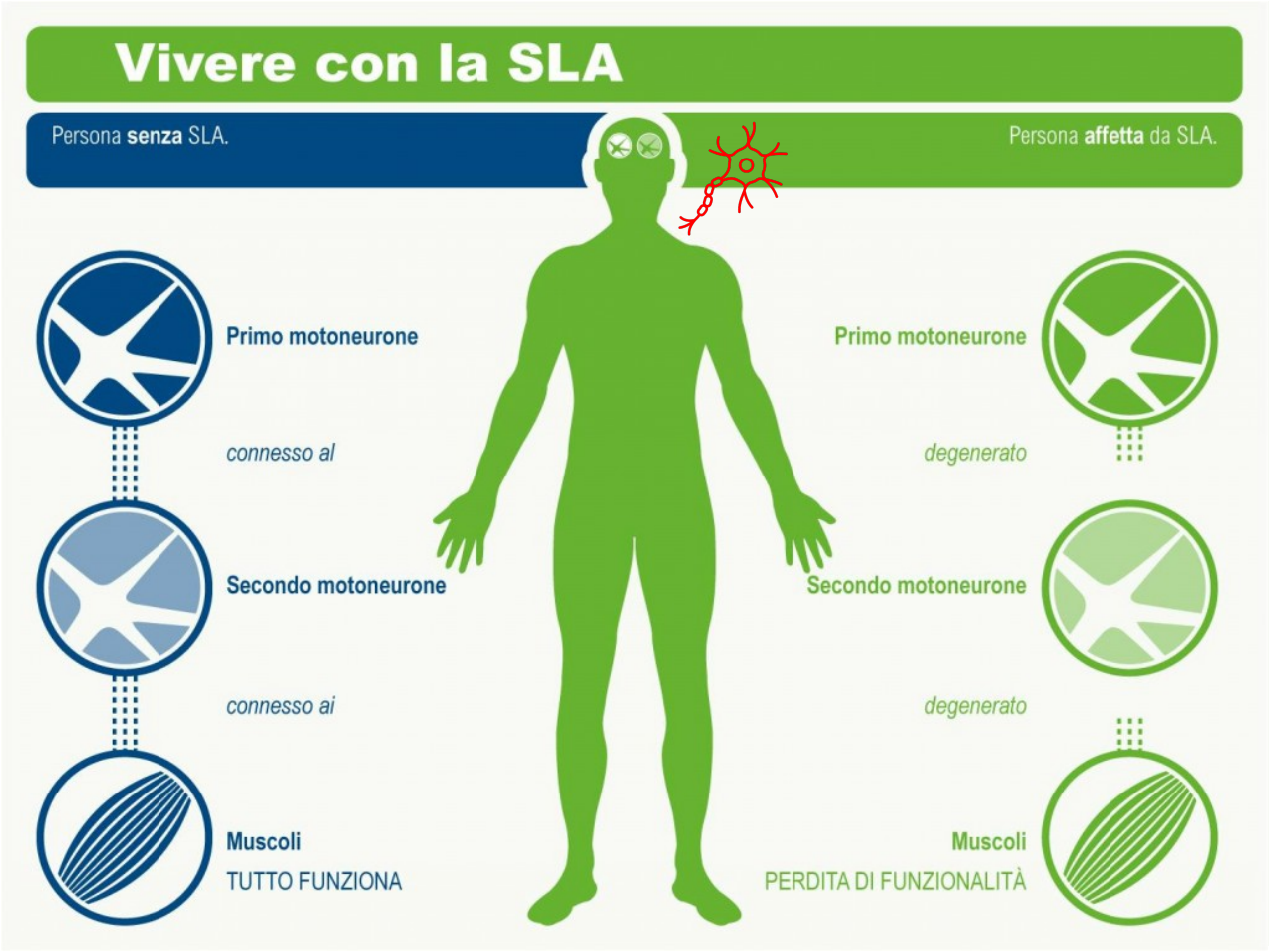
TITOLO TESI (INGLESE)

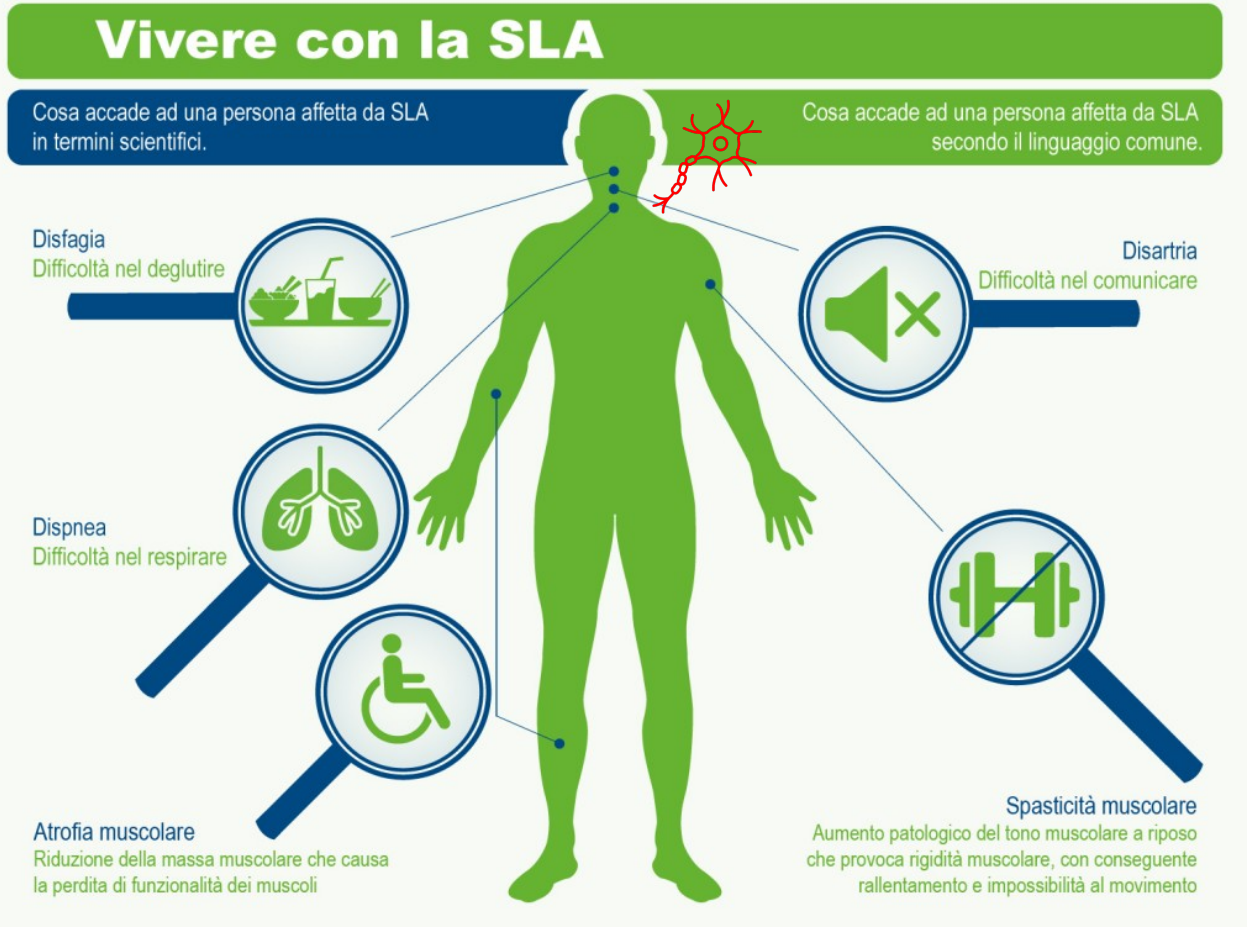
Oxidation-induced misfolding and aggregation of Superoxide Dismutase and its implications for Amyotrophic Lateral Sclerosis.

Sessione Estiva

Anno accademico 2022/2023

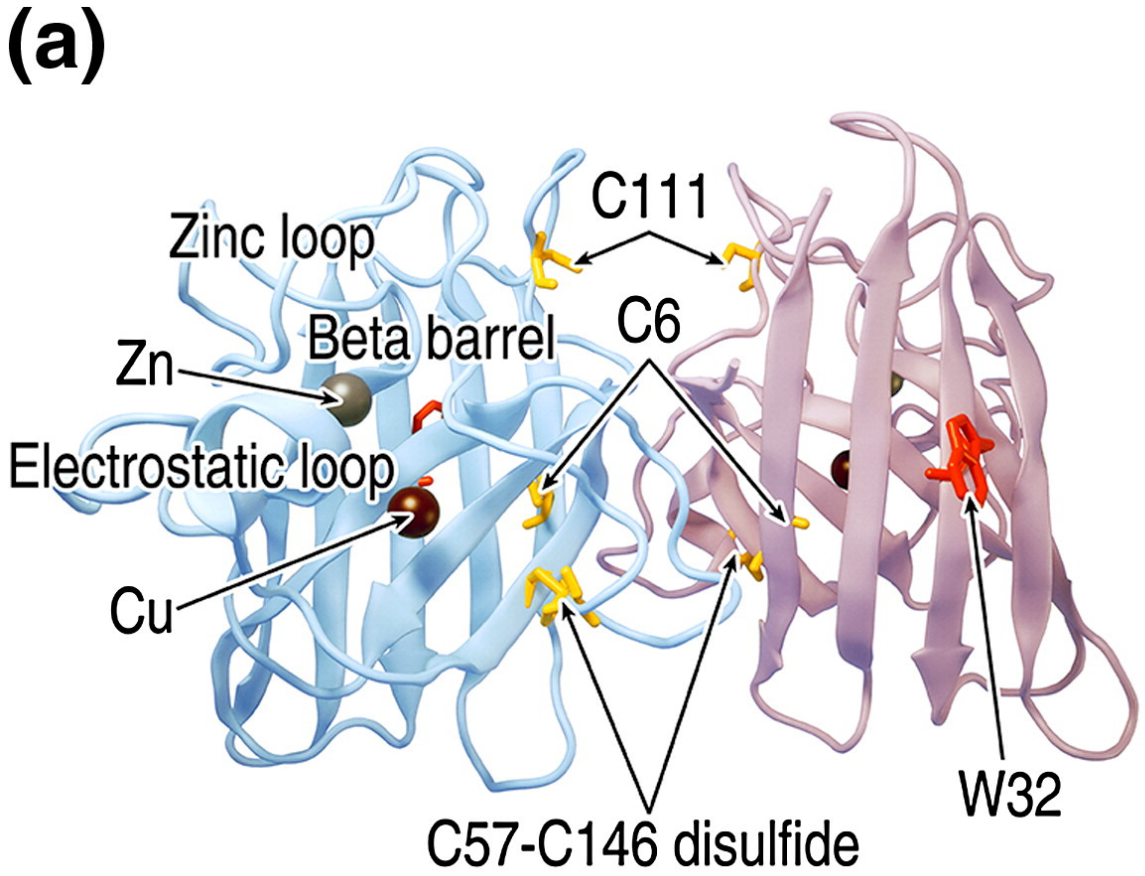
Sclerosi Laterale Amiotrofica

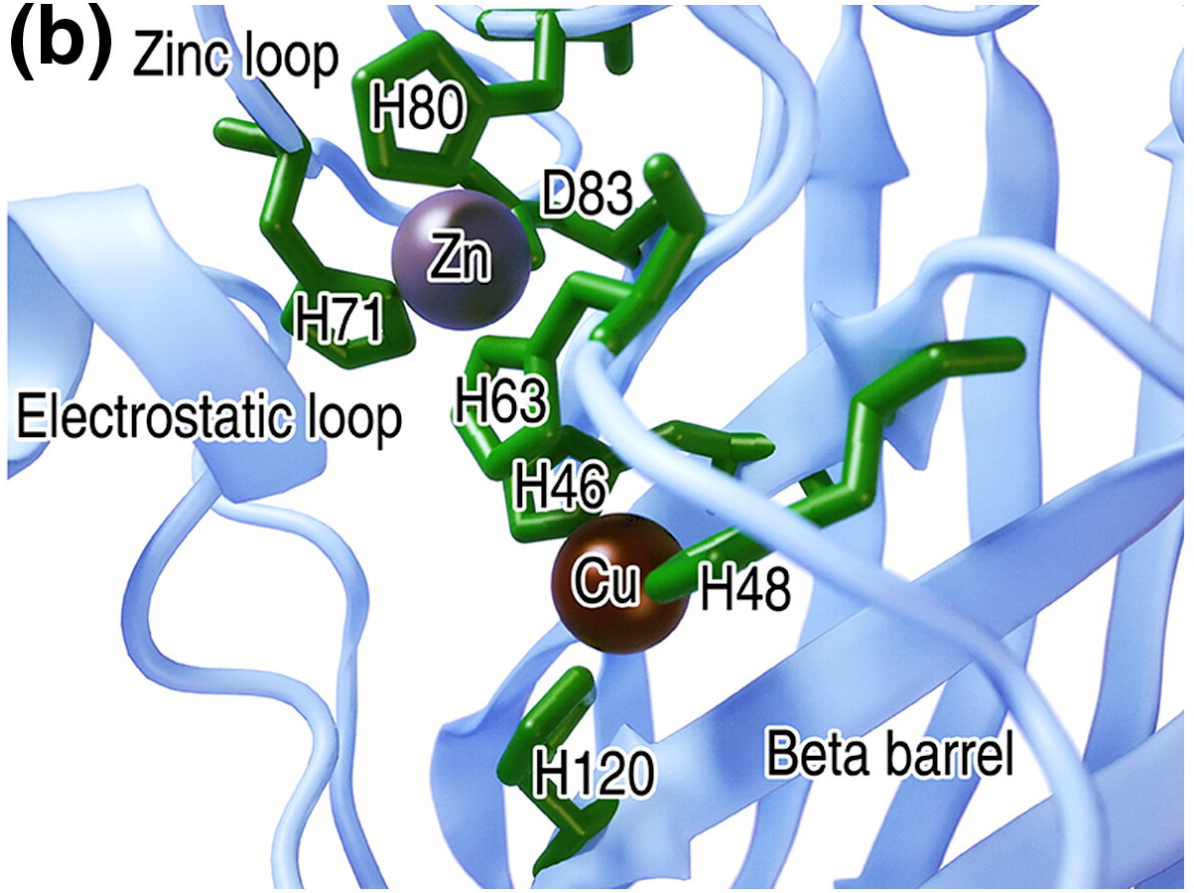




aisla ONLUS
ASSOCIAZIONE ITALIANA SCLEROSI LATERALE AMIOTROFICA

Cu/Zn Superossido dismutasi (wild-type)





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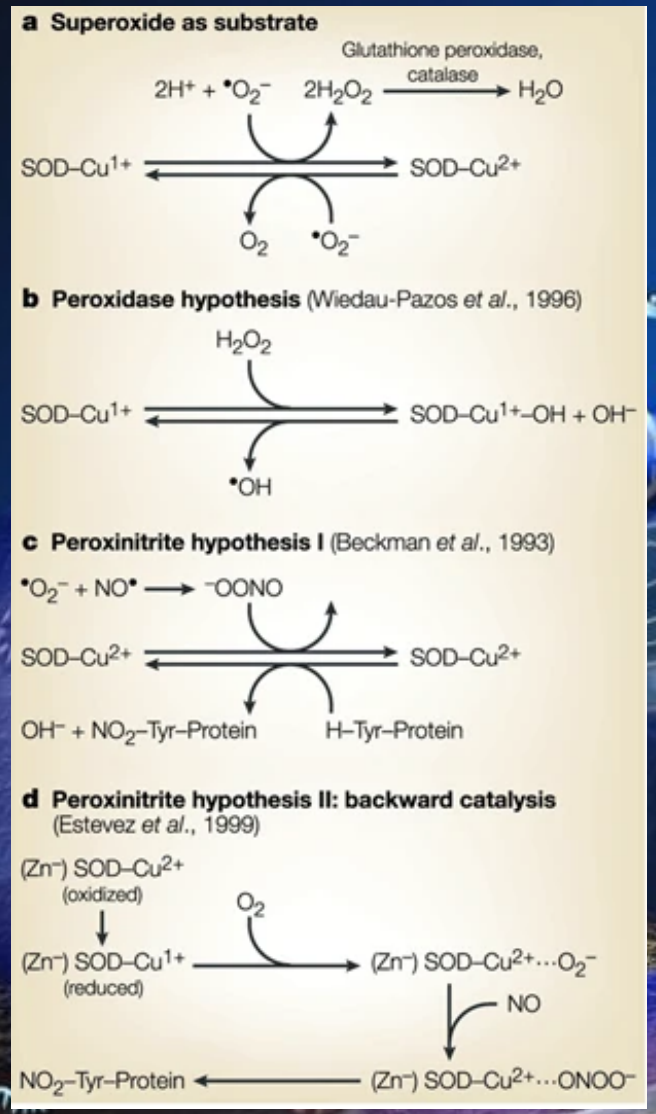
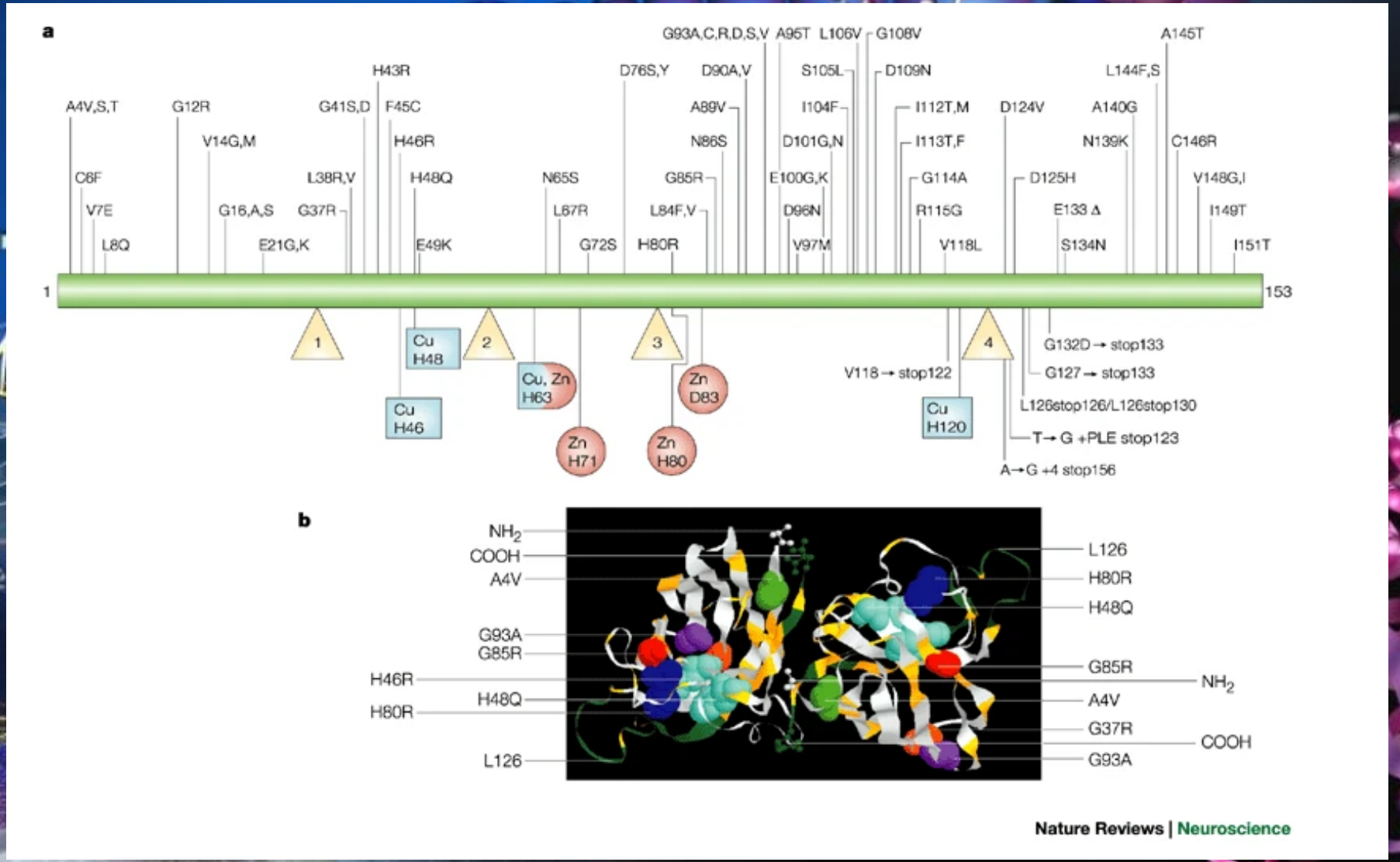
Mutazioni del gene *SOD1* e alterazioni enzimatiche





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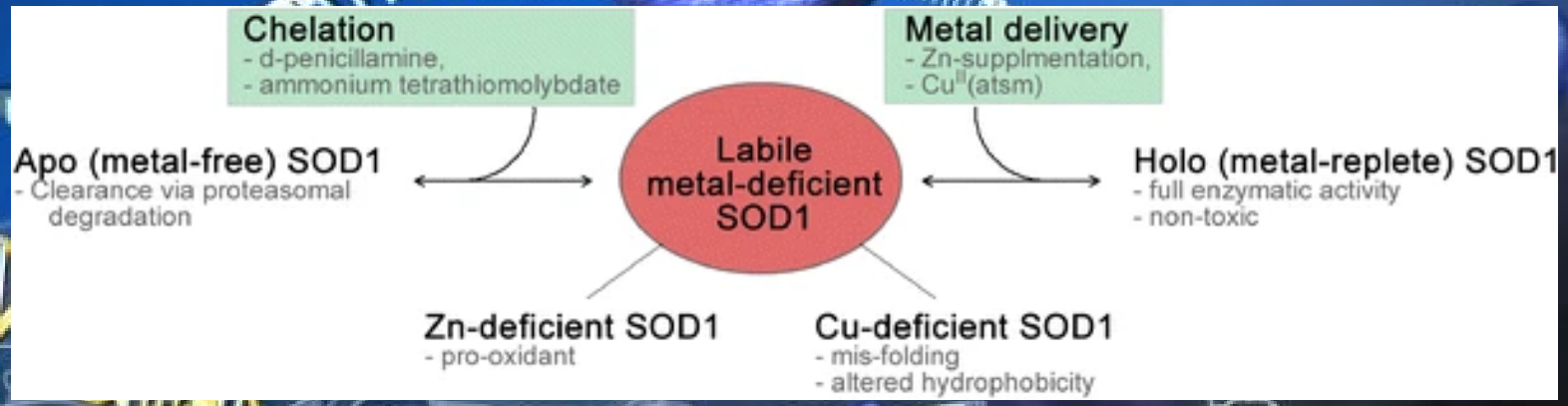
Modificazioni funzionali della Sod1



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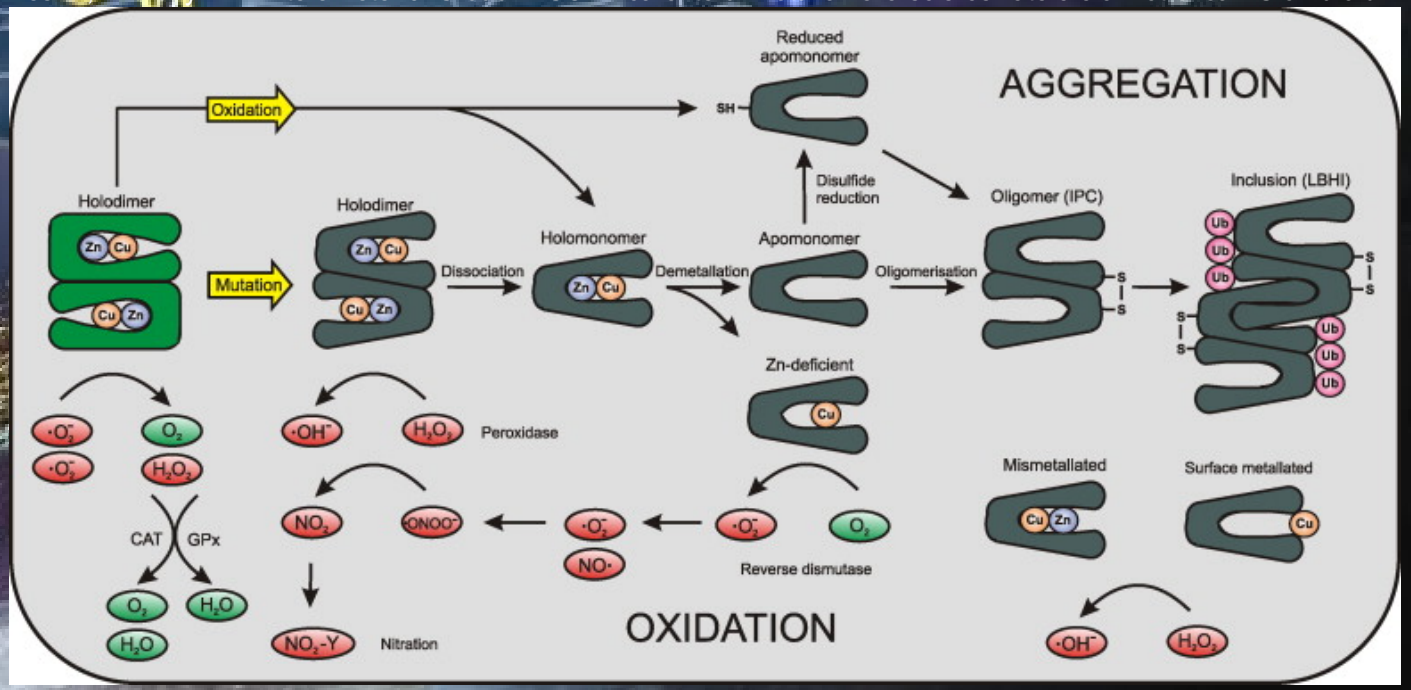

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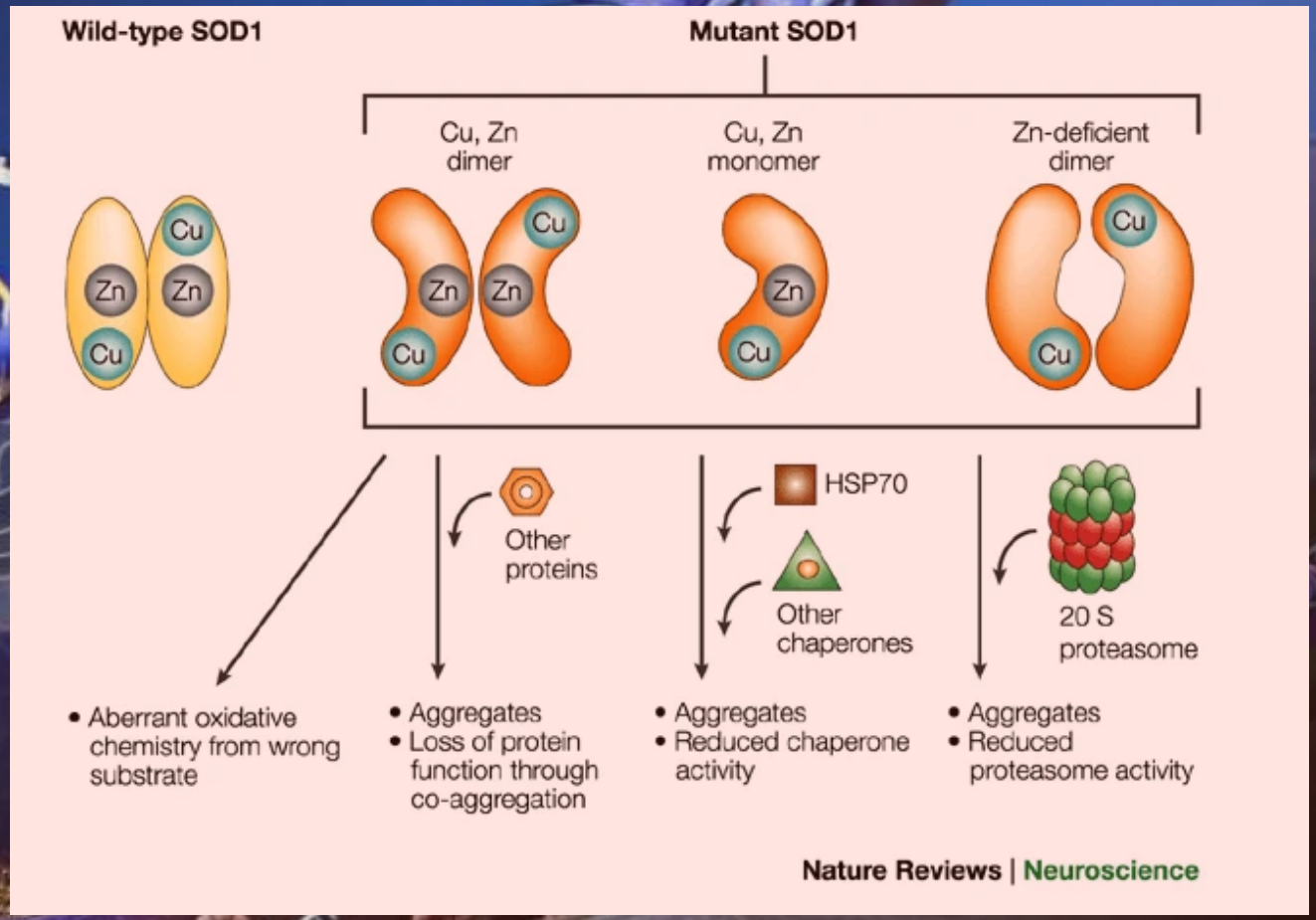
Conseguenze fisiologiche



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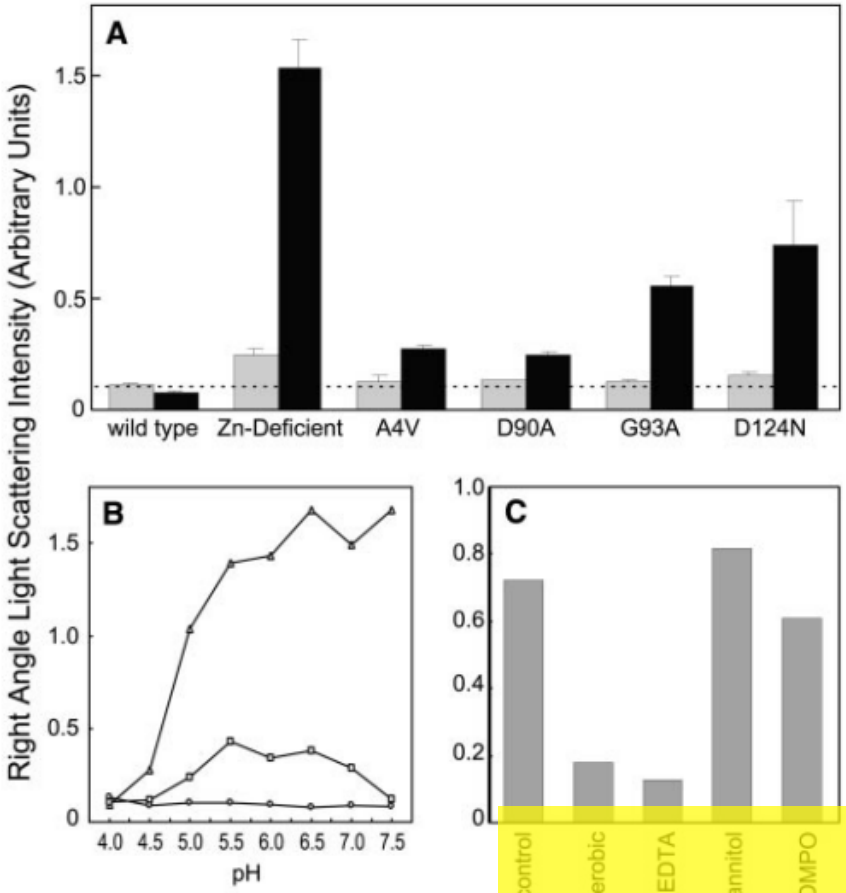
Sperimentazione



Tipologie di Sod1 utilizzate:

- Wild-type
- Zn-deficient
- D124N
- A4V
- D90A
- G93A

Ipotesi:

Ossidazione metallo-catalizzata sito-specifica

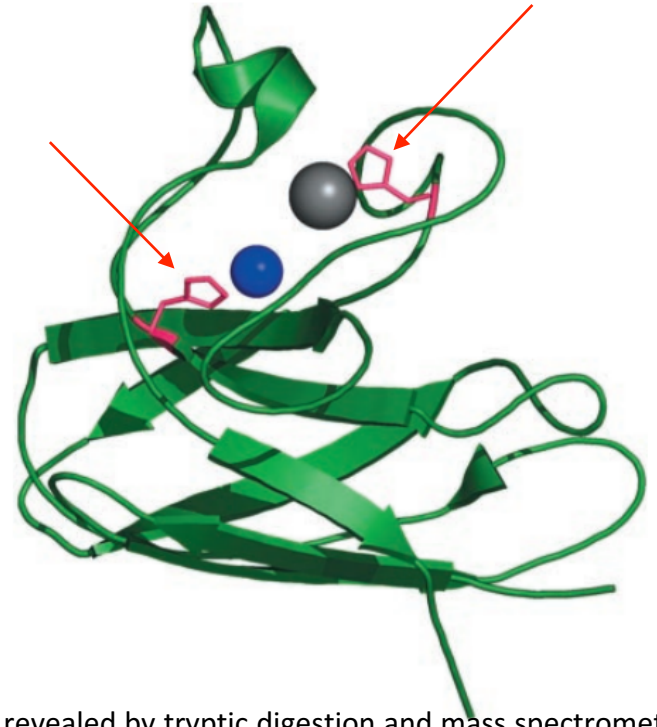
FIG. 1. Zinc-deficient and mutant SODs form visible aggregates on oxidation, whereas the wild-type protein does not. A, comparison of right-angle scattering signals from various SOD1 solutions on oxidation with 4 mM ascorbate and 0.2 mM CuCl2 (black) versus control (gray) at 37 °C, pH 7.0, for 48 h. Dotted line indicates scattering produced by 10 mM Tris acetate buffer with 4 mM ascorbate and 0.2 mM CuCl2. B, pH dependence of oxidation-induced aggregation of SOD. Zinc-deficient SOD1 forms visible aggregates over a large pH range (5.0–7.5) on oxidation (triangles). Wild-type SOD1 does not form visible aggregates under similar conditions (circles). Zinc-deficient SOD1 controls also yielded greater than base-line scattering (squares). C, lightscattering signal of zinc-deficient SOD1 treated with copper/ascorbate oxidation under various inhibition conditions (for details, see “Experimental Procedures”). Anaerobic conditions and copper removal by EDTA chelation prevents aggregation, whereas free radical scavengers (mannitol and DMPO) do not. Light-scattering measurements were made with a PTI QM-1 fluorimeter. Excitation and emission wavelengths were set to 350 nm (bandpass 2 nm).

Sito di modificazione dell'ossidazione

TABLE II.
Summary of mass spectroscopic analysis of tryptic fragments of oxidized SOD1

Tryptic peptide sequence	Position	Theoretical mass $[M + H]^+$	Experimental mass		Modified amino acid
			Control	Oxidized	
HVGDLGNVTADK	80–91	1225.6	1225.6	1225.6	His80
				1241.6	His80 + His16
TLVVHEK	116–122	825.5	825.5	825.5	His120
				841.5	His120 + His16

Aumento di 16 u.m.a.
per i residui:
Hys80 (Zn) e Hys120 (Cu)



FASTA

superoxide dismutase [Cu-Zn] [Homo sapiens]

NCBI Reference Sequence: NP_000445.1

GenPept Identical Proteins Graphics

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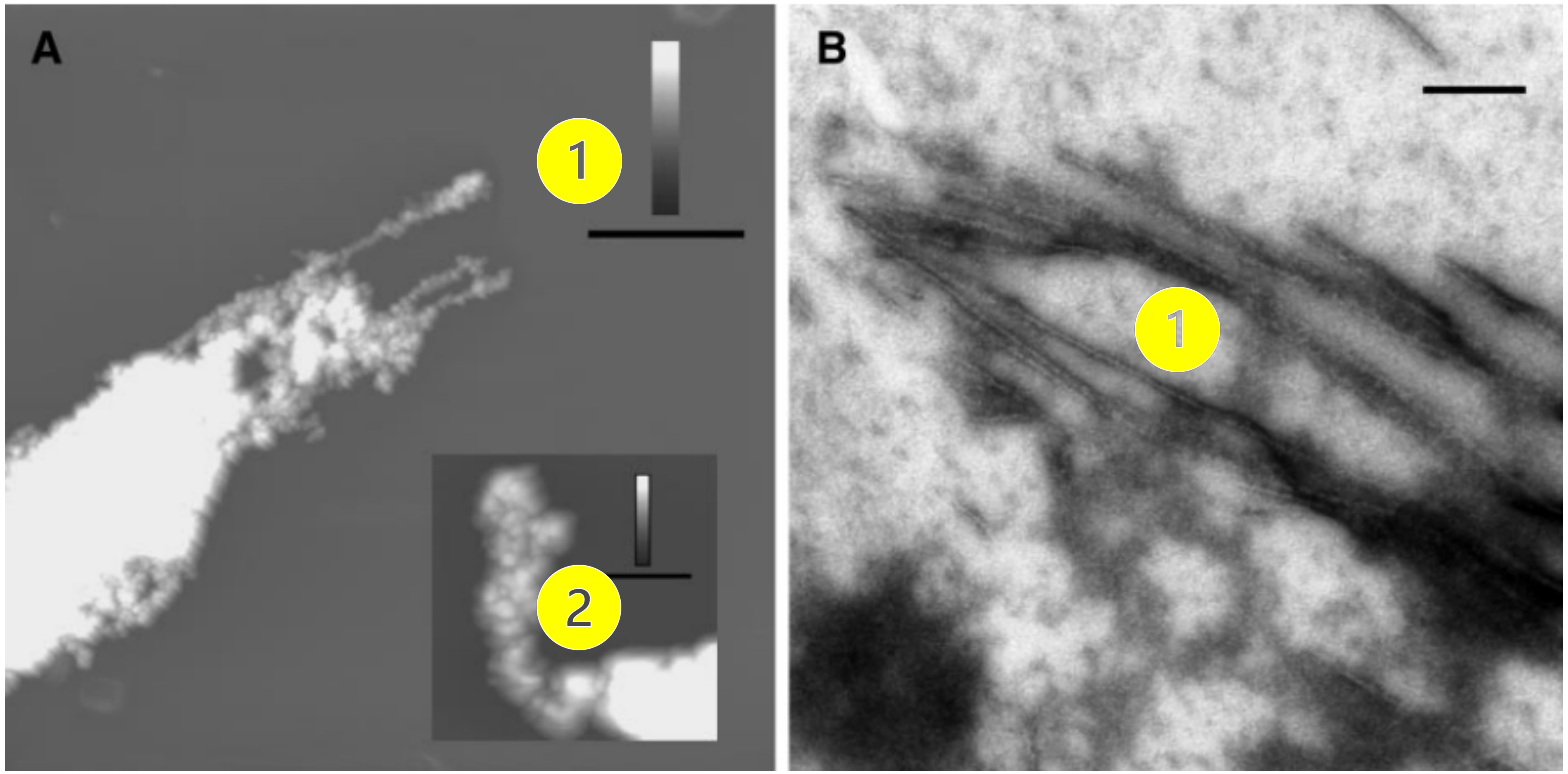
>NP_000445.1 superoxide dismutase [Cu-Zn] [Homo sapiens]
MATKAVCVLKGDGPVQGIINFEQKESNGPVKVWGSIKGLTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSR
KHGGPKDEERHVGDLGNVTADKDGVADVSIEDSVISLSGDHCIIGRTLVVHEKADDLGKGGNEESTKTGN
AGSRLACGVIGIAQ
```

FIG. 2. Oxidative modification sites of SOD1 revealed by tryptic digestion and mass spectrometry. SOD (30 M) was incubated with 2 mM ascorbate, 25 M copper, 10 mM sodium acetate, pH 5.0, at 37 °C for 24 h. The protein was reduced and alkylated with dithiothreitol and iodoacetamide in 6 M guanidine hydrochloride and then digested with trypsin (25:1 substrate to enzyme ratio) at 38 °C for 50 h and analyzed by capillary LC-MS/MS. The ribbon diagram was created from the PDB coordinates 1SPD with use of the program PYMOL (Delano Scientific). Side chains of modified His residues (80 and 120) are orange, the copper ion is blue, and the zinc ion is gray.

Morfologia degli aggregati proteici

L'aggregazione enzimatica porta ~~a fibre amiloidi~~ ?.....ad agglomerati amorfi

Oxidation-induced Aggregation of SOD1

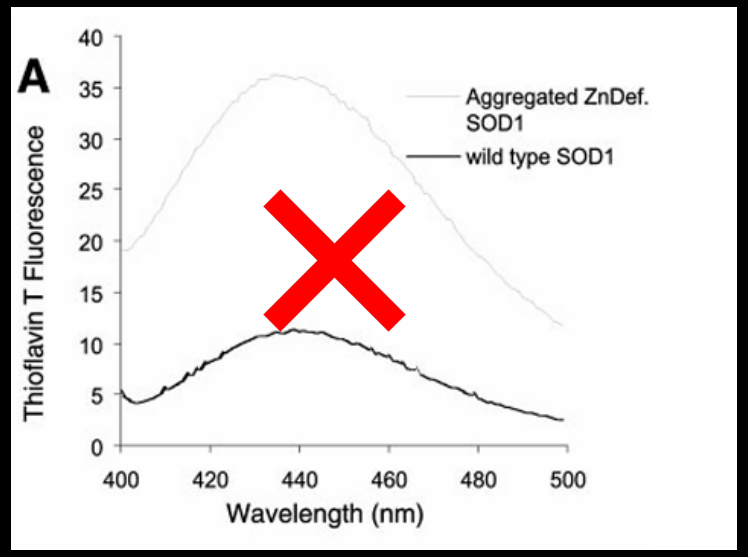


Differenze morfologiche con le placche amiloidi:

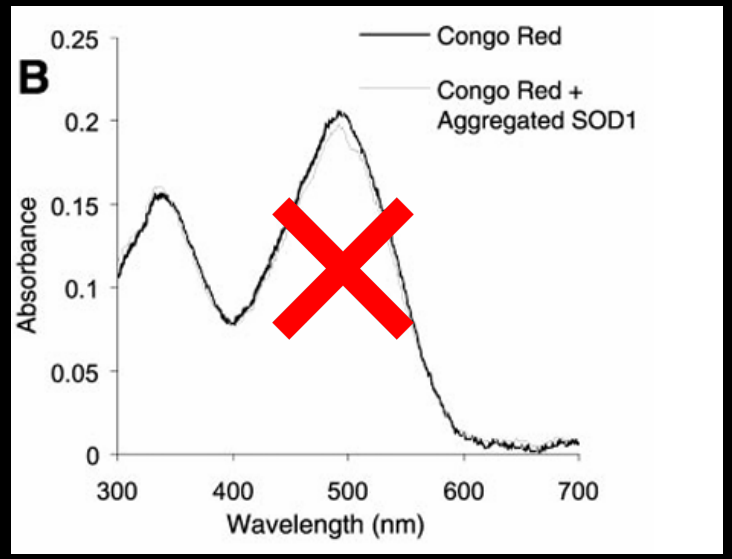
1. «fibre» di diametro maggiore
2. Corpuscoli più piccoli

FIG. 3. A, atomic force microscope height image of an aggregate formed by zinc-deficient SOD1. Aggregates are large and amorphous. Horizontal scale bar 10 m, vertical scale 2 m. Inset: close-up of protein aggregate shows that the aggregate is made up of smaller particles. Horizontal scale bar 2 m, vertical scale 1 m. B, transmission electron micrograph of SOD1 incubated in the presence of 25 M copper and 2 mM ascorbate in 10 mM sodium acetate buffer, pH 5.0, for 48 h at 37 °C. Scale bar 400 nm.

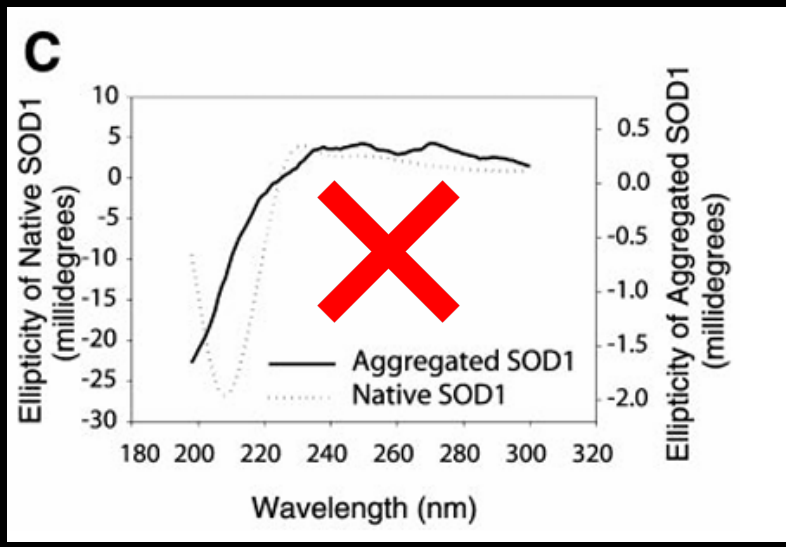
«Prova del 9»



A. Utilizzo della Tioflavina T



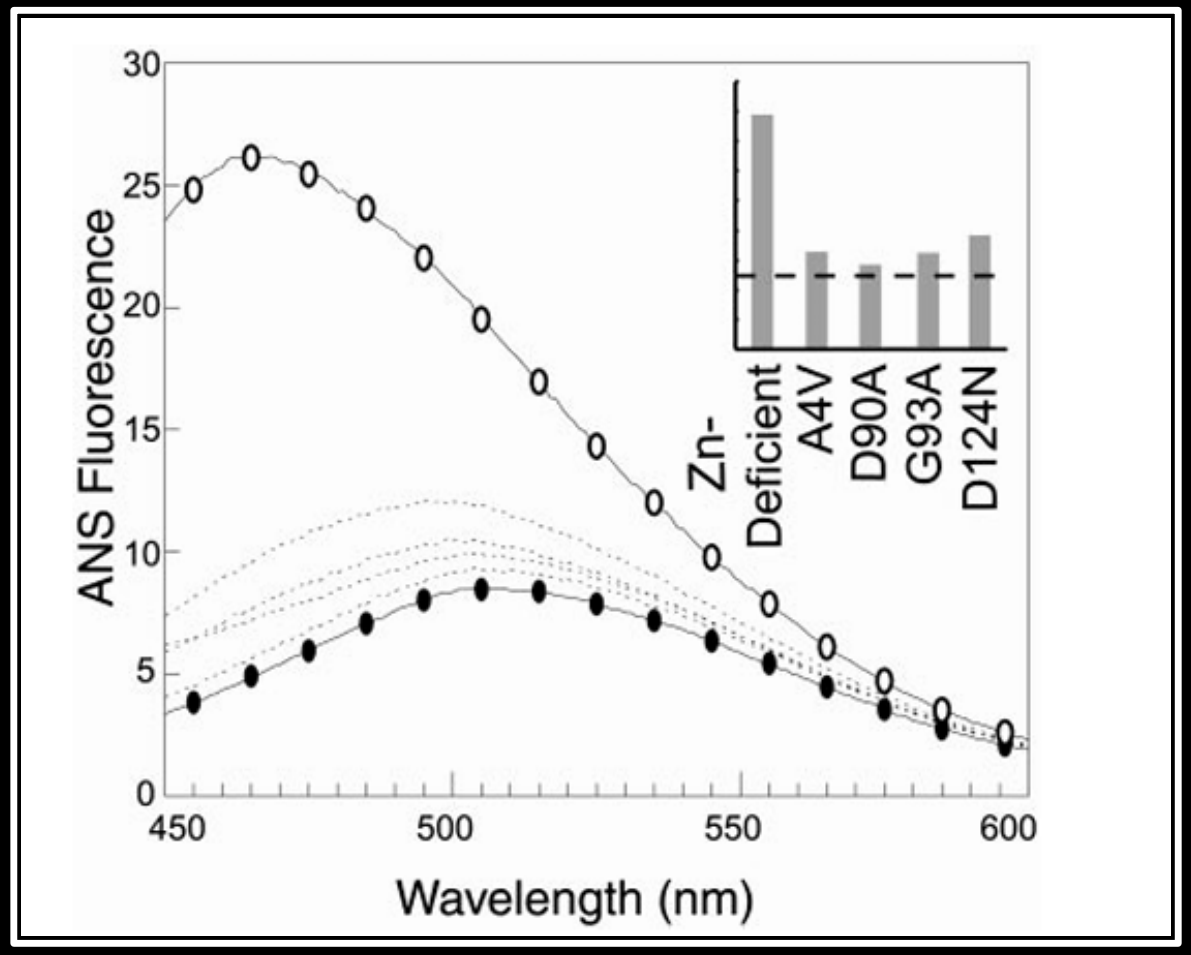
B. Utilizzo del colorante «Congo Red»



C. Spettro di Dicroismo Circolare

FIG.4
A, comparison of thioflavin T binding of zinc-deficient SOD aggregates (thin line) and wild-type SOD (thick line). Ten M SOD1 in 10 mM Tris acetate (pH 7.0) was incubated with 20 M thioflavin T before the emission spectrum was measured (excitation at 450 nm). Although some increase occurred in observed fluorescence intensity, it is far less than the increase typically seen on thioflavin T binding to amyloid fibrils. The increase observed is attributed to sequestering of the fluorophore from quenchers.
B, spectral shift assay of aggregates with the use of Congo red. Six M Congo red (solid line) had comparable absorbance to 3 MSODand6MCongored (dottedline).
C,CDspectra of SOD aggregates (solid line) and native SOD (dotted line). SOD aggregates do not contain a high proportion of-sheet structures and, with the dye binding experiments, this indicates that these aggregates are likely not amyloid.

Cambiamenti strutturali precedenti l'aggregazione



Utilizzo dell'acido 8-anilino-1-naftalensolfonico (ANS)

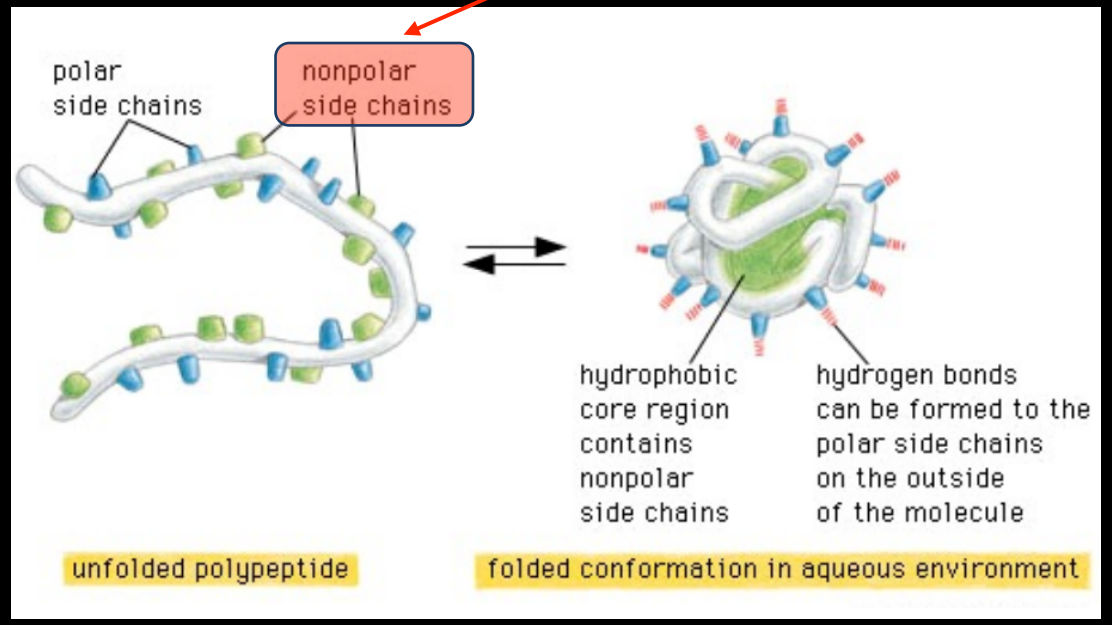


FIG.5. Comparison of ANS-binding of wild-type (filled circles) to mutant (dashed lines) and zinc-deficient SOD1 (open circles). Ten M SOD1 in 10 mM Tris acetate (pH 7.0) was incubated with 20 ANS before the emission spectrum was measured (excitation at 372 nm). Blue shift and increased intensity of ANS fluorescence in mutants and zinc-deficient SOD1 indicates increased exposure of hydrophobic domains. Inset: integrated ANS fluorescence signal from mutant and zinc-deficient SODs compared with ANS fluorescence of wild-type SOD1 (dashed line).

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Riassunto esteso

Lo studio pone in evidenza una fra le principali cause di sclerosi laterale amiotrofica: l'aggregazione intracellulare della Cu/Zn Superossido Dismutasi nei motoneuroni, ai vari livelli del Sistema Nervoso Centrale. L'emivita e la concentrazione dell'enzima, relativamente elevate in questi distretti, lo rendono suscettibile a danni ossidativi ad opera di «ROS». L'effetto è rappresentato dalla formazione di aggregati proteici dovuti ad eventi di misfolding casuale. Le mutazioni puntiformi sono responsabili delle modificazioni della sod1, con rispettive vairanti, alcune delle quali carenti in Zinco e particolarmente prone all'aggregazione spontanea a seguito di danno ossidativo. Il bersaglio del processo di ossidazione è il sito catalitico dell'enzima legante i metalli (Cu e Zn), composto da residui di istidina, che convertiranno in 2-ossistidina. Il meccanismo più plausibile alla base della formazione degli aggregati amorfi nei neuroni motori che sono causa di sla è dovuto al cambio di stato di metallazione dell'enzima.