Brief Summary

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Theme

Methylmercury (MeHg) Toxicity: Up-to-date Research on Mechanisms, Toxicology and Pathology

1) MeHg-toxicity and selenium
2) MeHg-toxicity in the central nervous system
3) Posttranscriptional or posttranslational modification of proteins in MeHg-cytotoxicity
4) Approach from chemical aspects of MeHg
5) Study on the MeHg-toxicity using small animal models
6) Effect of MeHg on vascular system
7) MeHg-toxicity and chemokine

1) MeHg-toxicity and selenium

1-1 Neuroprotection and Methylmercury: Selenium, DHA, and Nimodipine (Dr. Newland)

Sodium selenite

- **Developing rat model**
  No protection against brain function
- **Chronic adult-onset MeHg exposure**
  Delayed onset of MeHg-related signs

Protective effects of selenomethionine against methylmercury-induced neuronal degeneration in developing rat brain (Dr.Sakamoto)

Selenomethionine

- **Model rat of fetal-type Minamata disease (high dose)**
- **Biochemical & pathological studies**

Protection of brain cells against MeHg-induced neuronal degeneration

2) MeHg-toxicity in the central nervous system in vitro and in vivo

1-2 Effects of methylmercury on survival and differentiation of neural stem cells (Dr. Ceccatelli)

Neural stem cells (NSCs): mouse NSC line

rat primary embryonic cortical NSCs

highly susceptible to MeHg toxicity

1. MeHg-induced apoptosis

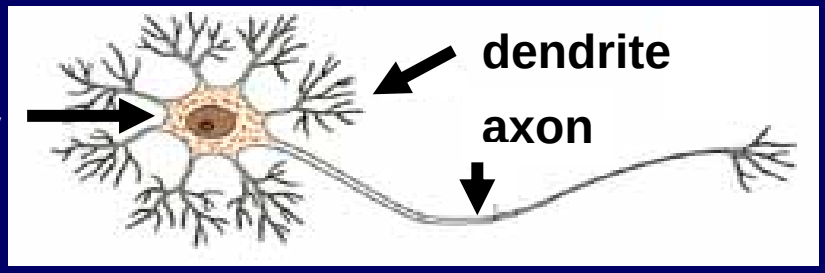
activation of Bax, cytochrome c release, activation of caspases and calpains

2. Impairment of neuronal differentiation via Notch signaling (2.5 or 5 nM)

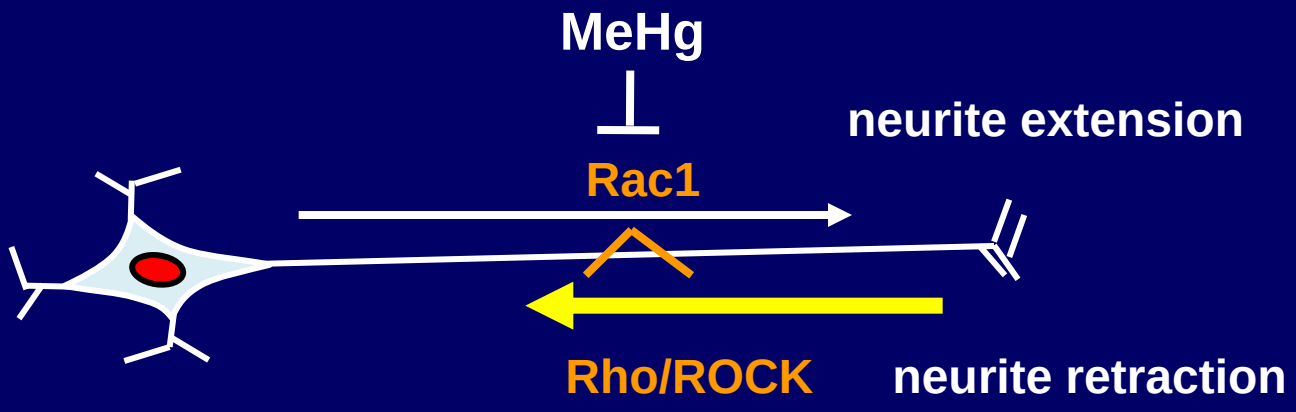
cf. PCBs promote instead neuronal differentiation.

7-1 Neuritic degeneration contributes to MeHg-induced neuronal cell death (Dr. Fujimura)

Cultured cortical neuronal cells



1. **Axonal degeneration contributes to MeHg-induced neuronal cell death.**
2. **Down-regulation of Rac1, which is known to promote neuritic extension, precedes MeHg-induced cortical neuronal damage.**



3. **Rho/Rock inhibitor (Fasudil and Y-27632) protected cells against MeHg-induced axonal degeneration and apoptotic neuronal cell death.**

7-2 Methylmercury activates multiple cell death pathways in neuronal and glial cells (Dr. Tofighi)

Cultured cells:

Neuron

- **Rat cerebellar granule cells (CGC)**
- **Mouse hippocampal neuron cell line**
- **Rodent pituitary tumor cell lines**

Glia

- **Human astrocytoma cell line**

Apoptosisi pathways:

- **mitochondrial/caspase-dependent pathway**
- **caspase-independent pathways**
  - **Ca^{2+}/calpain pathway**
  - **involvement of lysosomal enzymes such as cathepsins;**
  - **translocation of AIF (apoptosis inducing factor) into the nucleus**

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Oxidative stress plays a critical role in the onset of MeHg toxicity

Cross-talk between the various pathways activated concomitantly

6-1 Early changes of astrocytes in the molecular layer of cerebellar cortex of rats with methyl mercury intoxication (Dr. Izumo)

- **Cerebellum in MeHg-intoxicated model rat**
- **Neuropathological study:**
  histopathology, immunocytochemistry, electron microscopic studies

molecular layer of the cerebellum

- **increase of Iba1-positive activated microglia**
- **intense staining of GFAP immunohistochemistry in Bergmann's glia**
- **many small vacuoles**
  1. **swollen astrocytic processes with formation of vacuoles and accumulation of dense lamellar bodies**
  2. **normal morphological structure in Purkinje cell dendrites and pre- and post synapses**

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primary involvement of astrocytes in acute phase of MeHg intoxication

6-2 The role of glia in modulating MeHg neurotoxicity (Dr. Aschner)

Cultured cells: microglia, astrocyte

Microglia: the early responders following MeHg treatment

- **a lower basal GSH pool and a significantly greater Hg accumulation than astrocytes**
- **more susceptible to MeHg than astrocytes**
- **rapid generation of ROS**
  second messengers to amplify the pro-inflammatory function
- **activation of Nrf2**

Astrocytes: taking on a role at a later stage

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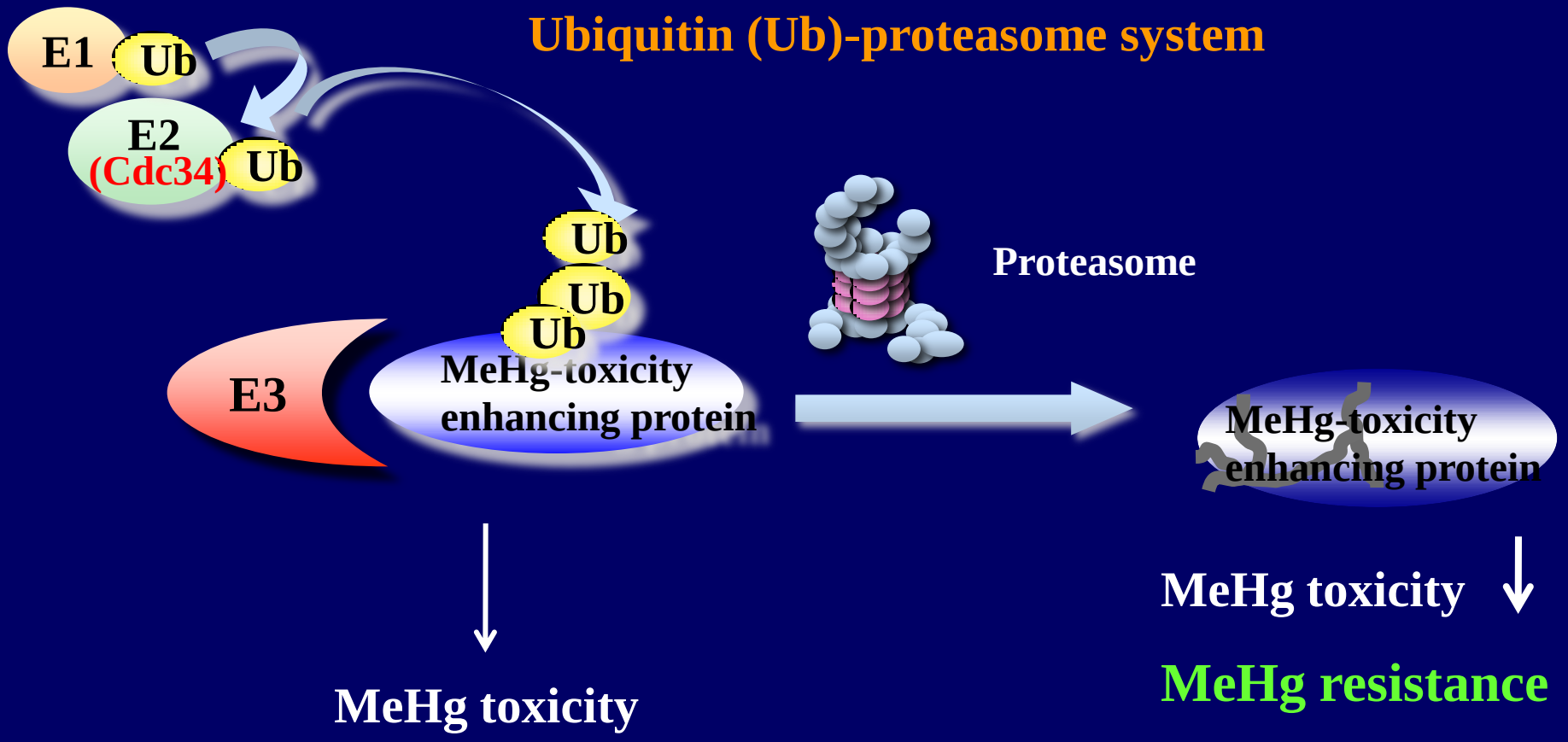
Microglia is the first line of cellular defense against MeHg toxicity in the CNS.

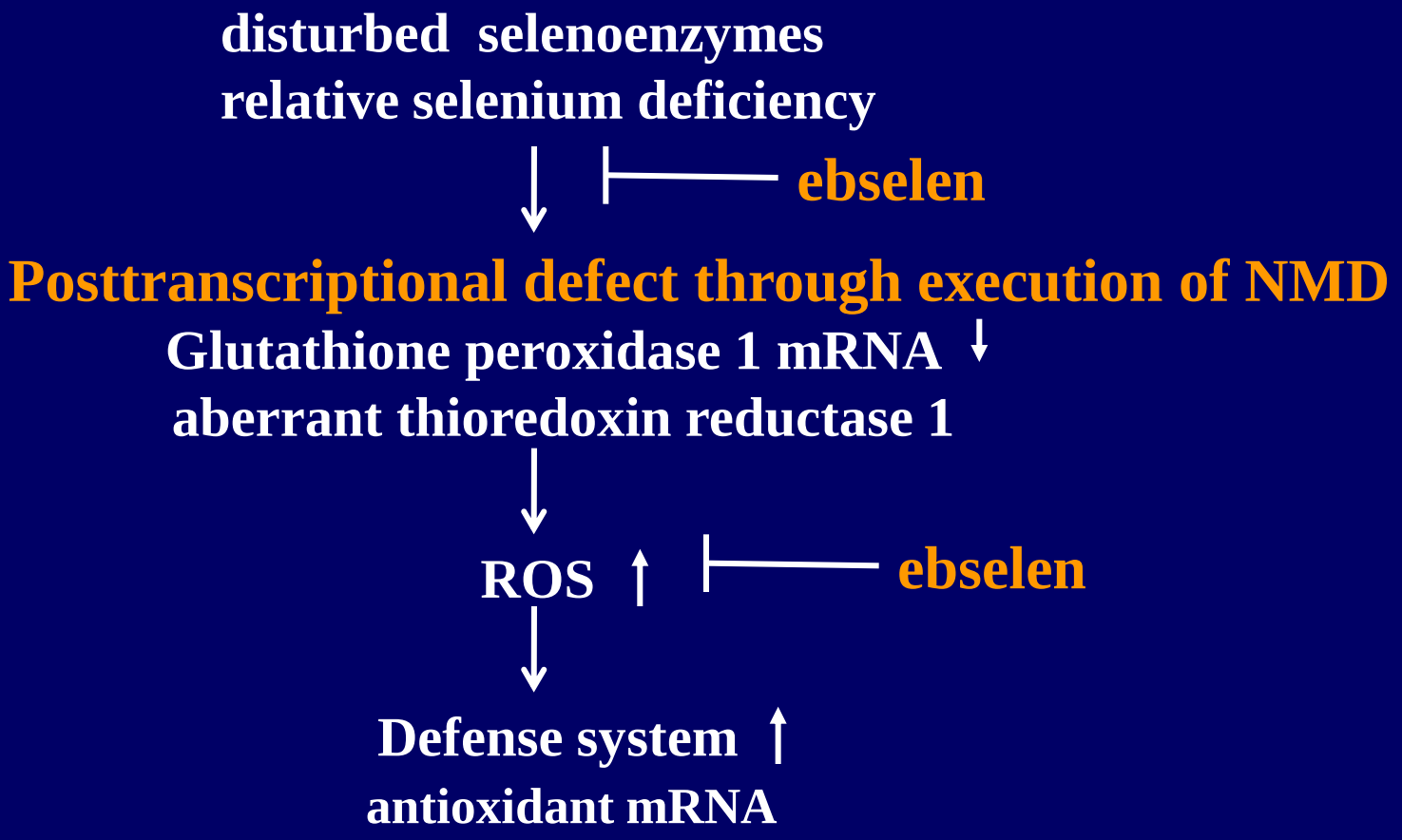
3) Posttranscriptional or posttranslational modification of proteins in MeHg-cytotoxicity

3-1 Involvement of the post-translational modification of proteins in MeHg toxicity (Dr. Hwang)



3-2 Posttranscriptional defects of antioxidant selenoenzymes cause oxidative stress under methylmercury exposure (Dr. Usuki)

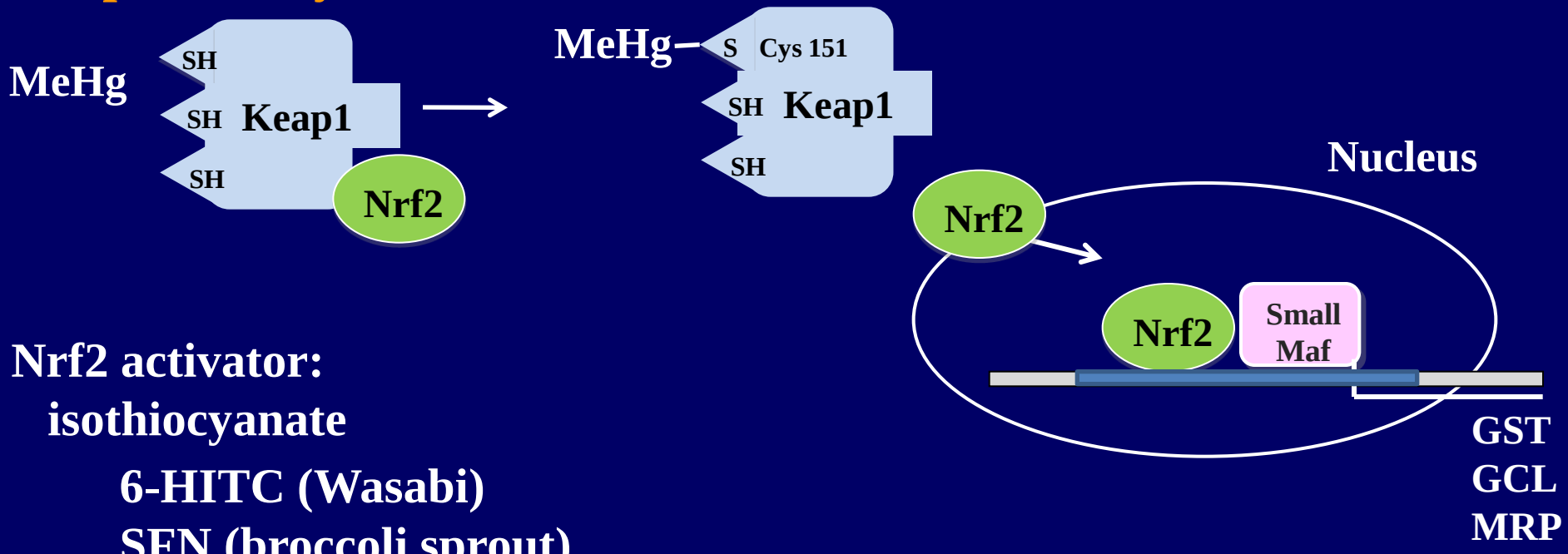
Incidence of oxidative stress following MeHg exposure



4) Approach from chemical aspects of MeHg

2-1 Keap1/Nrf2 system regulates cellular accumulation of MeHg, thereby blocking its toxicity (Dr. Toyama)

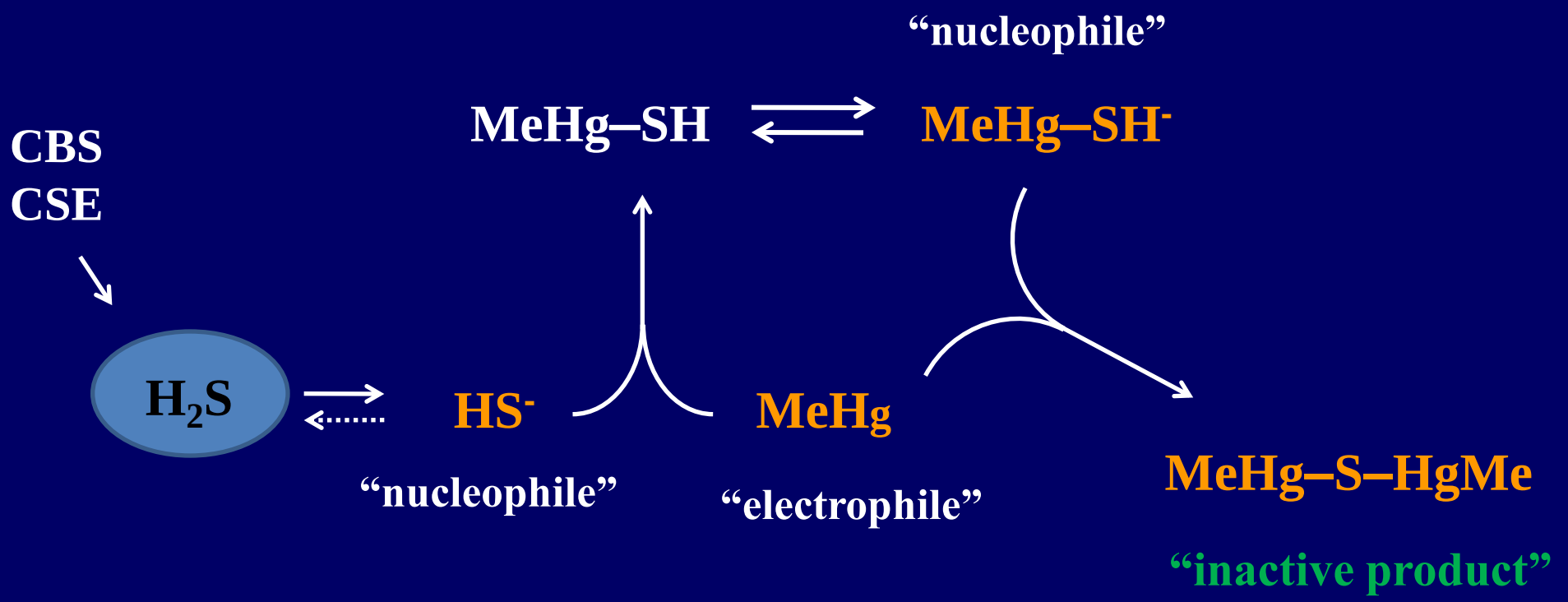
Keap1/Nrf2 system



Nrf2 activator:
isothiocyanate
6-HITC (Wasabi)
SFN (broccoli sprout)

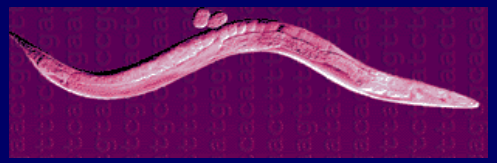
Excretion of MeHg
Suppress of MeHg toxicity

2-2 A unique protein mediating cellular protection against MeHg (Dr. Kumagai)



5) Study on the MeHg-toxicity using small animal models

5-1 Methylmercury neurotoxicity in a C. elegans model syste (Dr. Martinez-Finley)



C. elegans

Useful model system to investigate MeHg-toxicity

1. Protective protein against MeHg-toxicity

- **Glutathione**
- **Heat shock protein**
- **Skn-1: *c. elegans* orthologue of mammalian Nrf2**
- **Metallothioneine**

2. Vizualized neurons

DAergic neuron

GABAergic neuron

5-2 Zebrafish as a model of the mercurial contamination of the aquatic food web: histological, bioenergetical, and transcriptional issues (Dr. Bourdineaud)

Effect of dietary MeHg exposure <u>at environmentally relevant doses</u> :

1. **Modification of gene expression pattern in muscles and brain**
   oxidative stress, ER stress, mitochondrial damage,
   and detoxification.
2. **Damage in muscle mitochondria**
   inhibition of respiration, structural abnormalities
3. **Damage in optical tectum in brain**
   Decrease in nucleus areas in granular cells
   lower density of cells
4. **Decrease in hatching and the viability rate of the eggs**

6) Effect of MeHg on microvascular pericytes and endothelial cells

4-2 The microvascular cells are a target of methylmercury toxicity (Dr. Hirooka)

Cultured pericytes and endothelial cells

1. **Pericytes: more sensitive to MeHg than endothelial cells**
2. **Hyperpermeability in pericytes and endothelial cells**
   upregulation of VEGF system proteins
3. **Water accumulation in the extracellular matrix of vascular tissue**
   increased secretion of hyaluronan

7) MeHg-toxicity and chemokine

4-1 Protection by the chemokine CCL2/MCP1 of MeHg neurotoxicity (Dr. Rostene)

Chemokine CCL2 and its receptor CCR2:
produced by neurons and glial cells (in particular microglia)

Primary cortical neuronal cell culture and CCL2 knock-out mice

- **MeHg-mediated increase in CCL2 in cortical neuronal cell culture**
- **increase in neuronal cell death under block of CCL2 and CCR2**
- **pronounced neuronal cell death in CCL2 knock-out mice**

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CCL2 released by neurons allows activation of neighbouring microglia to produce CCL2 to protect neurons in the early phase of MeHg-cytotoxicity

Effect of materials on MeHg-toxicity

1. **Selenium**
   - **Selenomethionine**
   - **Sodium selenite**
2. **Calcium channel blocker**
   - **Nimodipine (dietary)**
4. **Nrf2 activator**
   - **Isothiocyanate**
     - **6-HITC, SFN**
5. **Seleno-organic compound**
   - **Ebselen**
6. **ROCK inhibitors**
   - **Fasudil, Y-27632**
7. **Chemokine**
   - **CCL2/MCP1**