Hippocrates Was Right

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- Let Food Be Your Medicine and Medicine Your Food

We Are in the Third era of Nutritional Research

- **First era:** Understanding the roles of carbohydrates, proteins & lipids
- **Second era:** Understanding the roles of vitamins & minerals
- **Third era:** Understanding how certain components of our diet (mainly phytochemicals) influence cellular signalling & gene expressions and, thus, health

Oxidative Stress Underlies Aging

Rembrandt's 'Apostle Paul'

- Oxidation (i.e., rusting) of molecules

- We rust as we age

Oxidative Stress

- **Direct Effect:** Through oxidation of critical signalling molecules it disrupts normal cell signalling and thereby alters gene expression that in turn leads to disease
- **Indirect Effect:** Oxidation of some of the signalling molecules promotes non-infectious inflammation
  - Non-infectious inflammation is a causal factor for Alzheimer's disease, atherosclerosis, kidney disease, obesity, vascular dementia, etc

- **Decreasing oxidative stress ought to result in a more graceful and healthier old age**

Cell Signalling

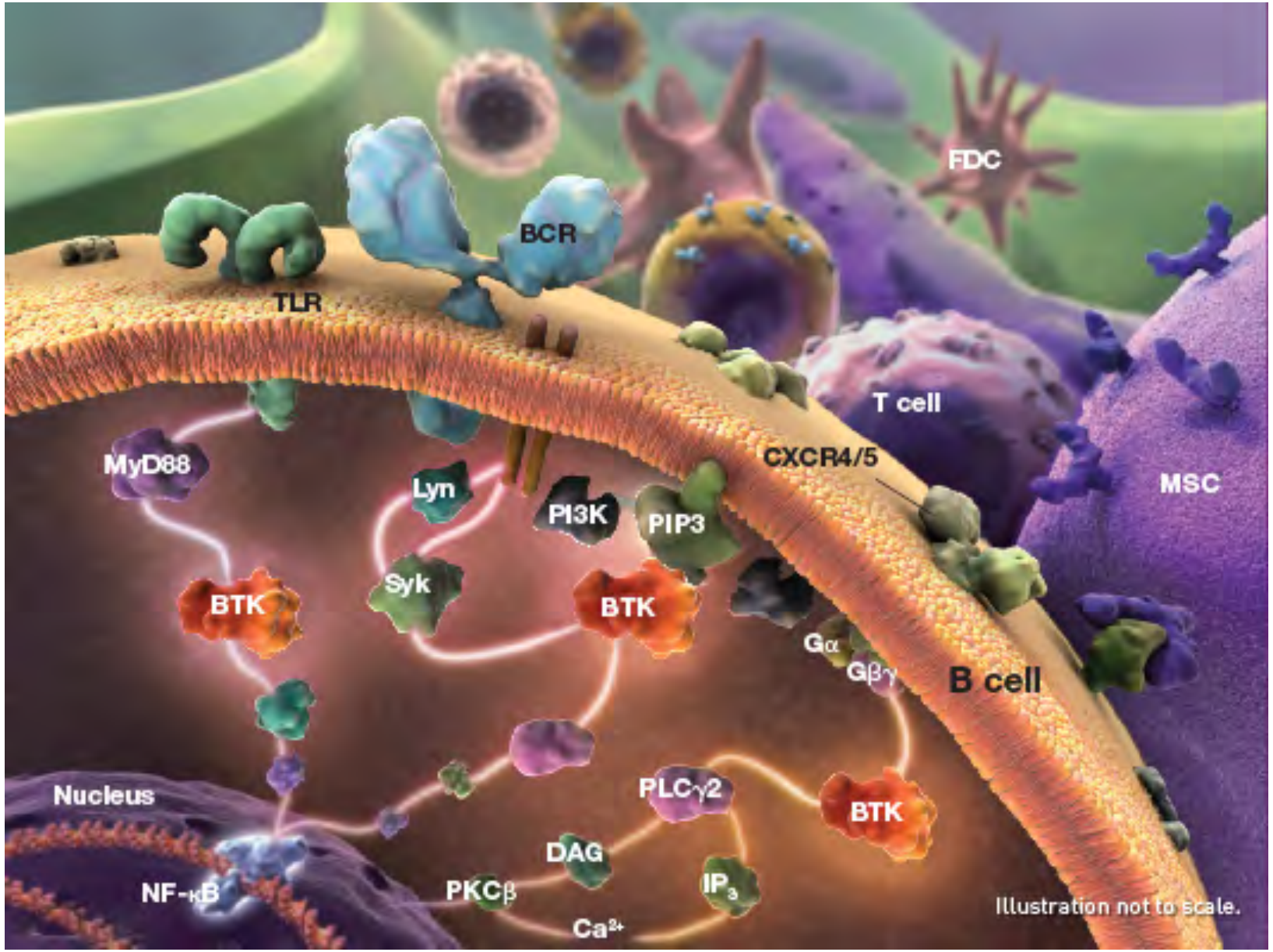


Image taken from: http://www.bcellmalignancy.eu/en/category/b-cell-signalling

What Is Cell Signalling? Cell Signalling Can Be Likened To A Cell Phone Network

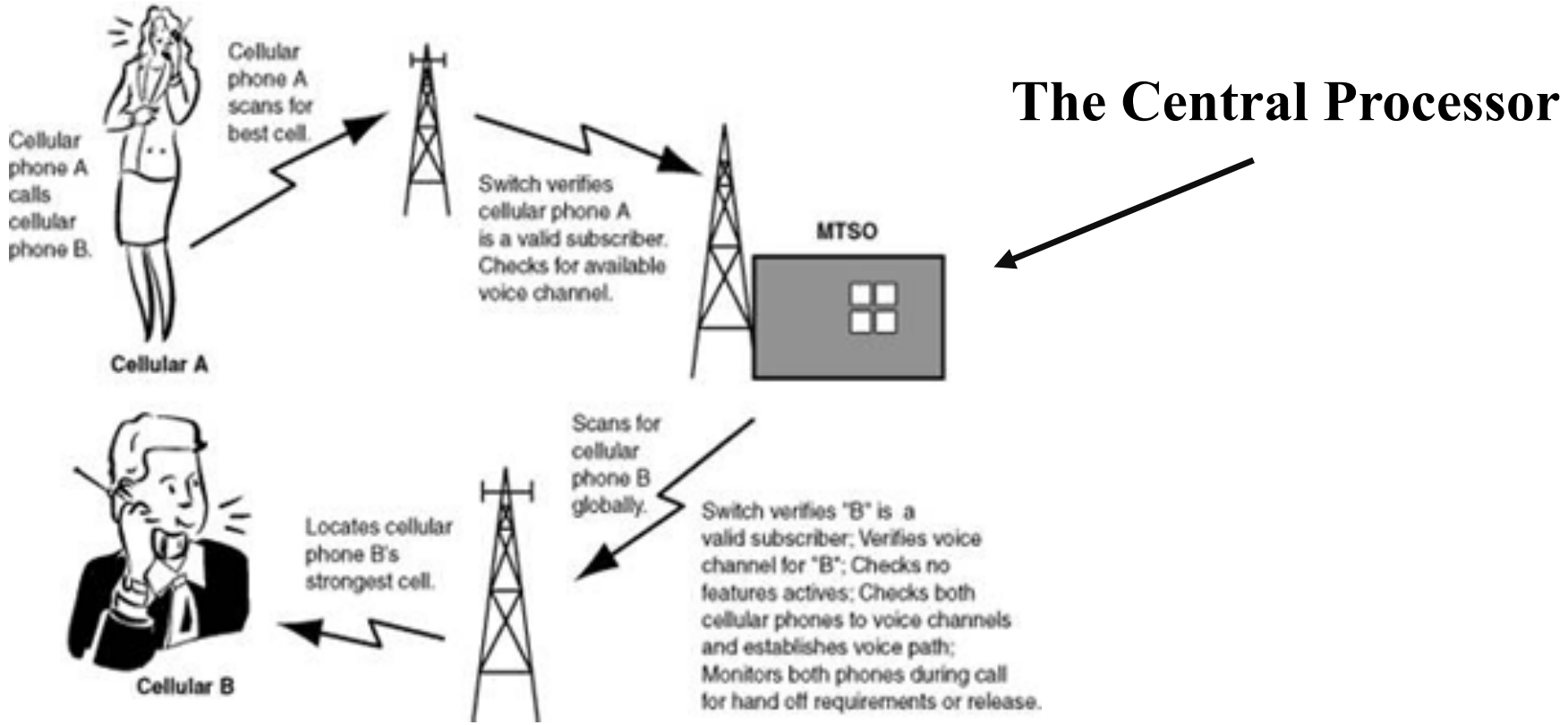


Image from: http://www.electroschematics.com/5231/mobile-phone-how-it-works/

Cell signalling requires a signal sender, signal relayer & amplifier, analyser & responder and an outcomes component

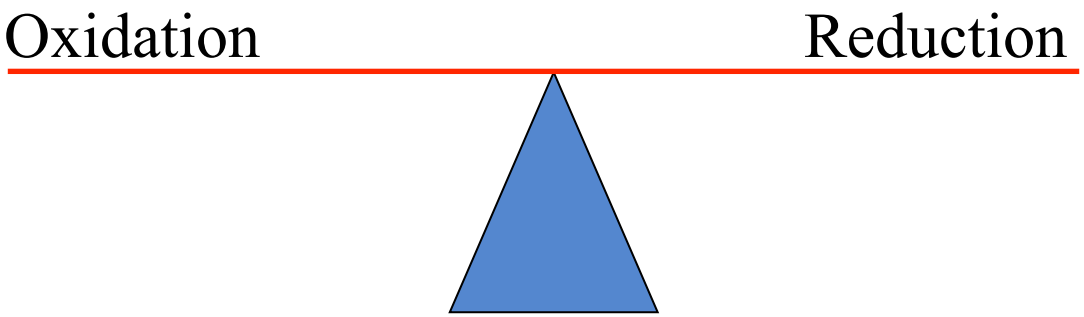
Oxidation & Reduction

- Oxidation: loss of electrons by a component
- Reduction: gain of electrons by a component

- These are referred to as redox reactions

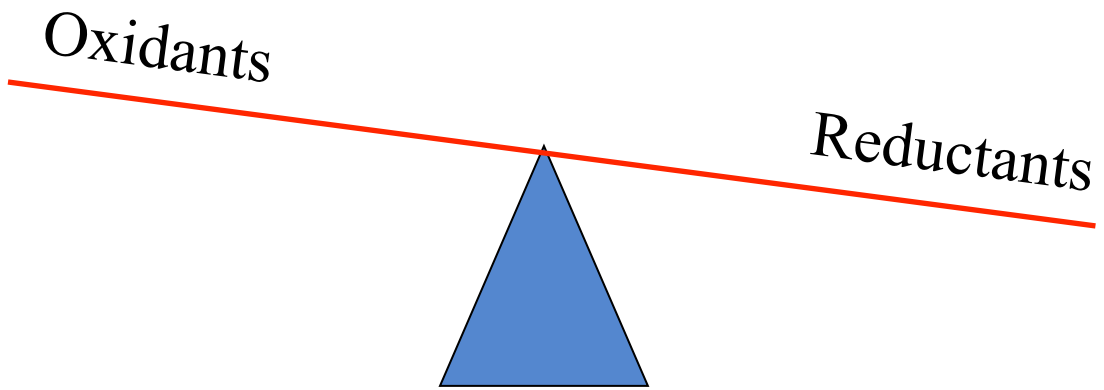
Reduction-Oxidation (Redox) Reactions

- Our metabolism produces oxidants and reduces (inactivates) the oxidants produced
- When we are young this oxidation and reduction is balanced



Oxidant-Inactivating Mechanisms

- Have decreased expression with age
  - With healthy aging there is a slower decline, but a decline, nevertheless, occurs
- **But** expression of oxidant-inactivating mechanisms can be increased using a dietary approach and this should lead to healthier aging



How To Counter Aging-Related Oxidative Stress

- Dietary anti-oxidants are not the answer
  - We can never consume enough anti-oxidants to have an impact
  - One anti-oxidant can inactivate one oxidant but trillions of oxidants are produced each hour of metabolism
- We need to to enhance the **endogenous cellular mechanism** that inactivate oxidants and/or decrease the production of strong oxidants

The Nrf2 Signalling System

- Activation of the protein known as Nrf2 results in increased expression of dozens of genes whose protein products:
  - Decrease the probability of strong oxidants being produced
  - Increase the mechanisms that inactivate oxidants
  - Increase the functioning of mitochondria, the power plants of the cell that produce the cellular fuel, ATP

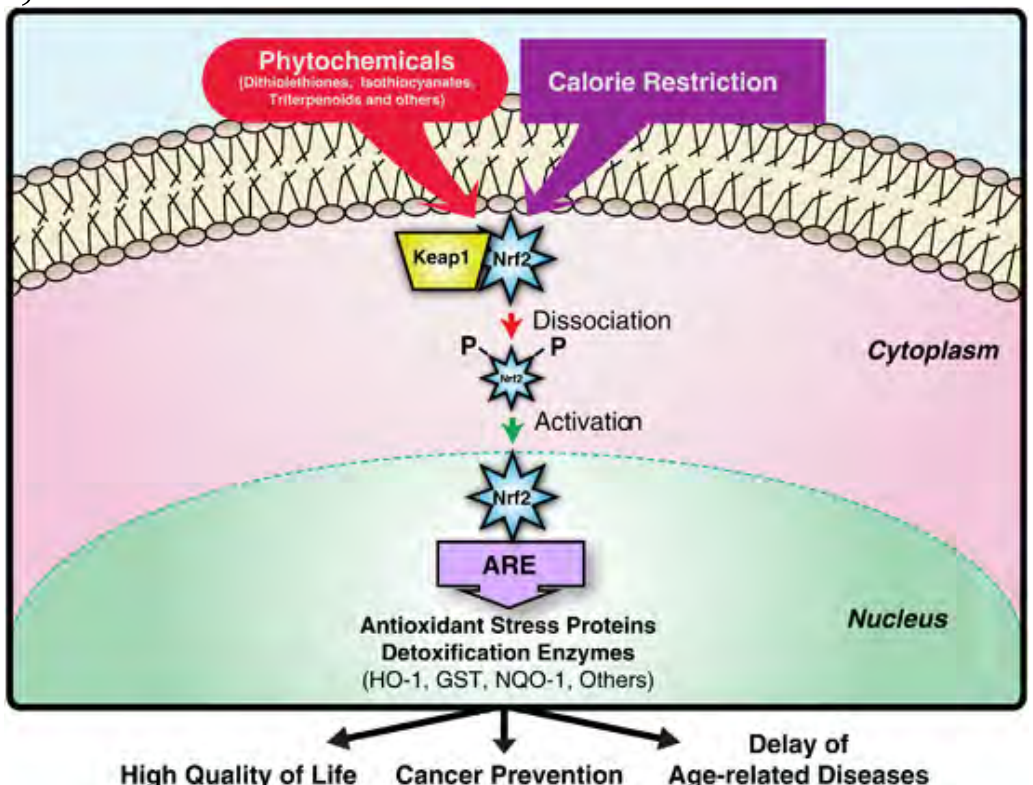


Image taken from: Martin-Montalvo *et al.* 2011. *Oncogene* **30:** 505-520

The Nrf2 Signalling System Continued

- There are certain components in our diet, mainly phytochemicals, that activate the Nrf2 signalling system
  - Many of the protein products of the genes induced are collectively referred to as phase 2 enzymes that are involved in the inactivation of many chemicals such as carcinogens






Nrf2 Activators

- Discovery of the Nrf2 signalling pathway involved many researchers from around the world. Some of whom are:
  - Lee Wattenberg and colleagues of the University of Minnesota described how certain chemicals that induce the expression of the phase 2 drug metabolizing enzymes could inhibit chemically-induced cancers. The specific pathway was still unknown.
  - Thomas Rushomore & Cecil Pickett of the Merck Frosst Centre, Quebec who discovered the Anti-Oxidant Response Element (ARE) in the promoter region of "phase 2 enzyme genes"

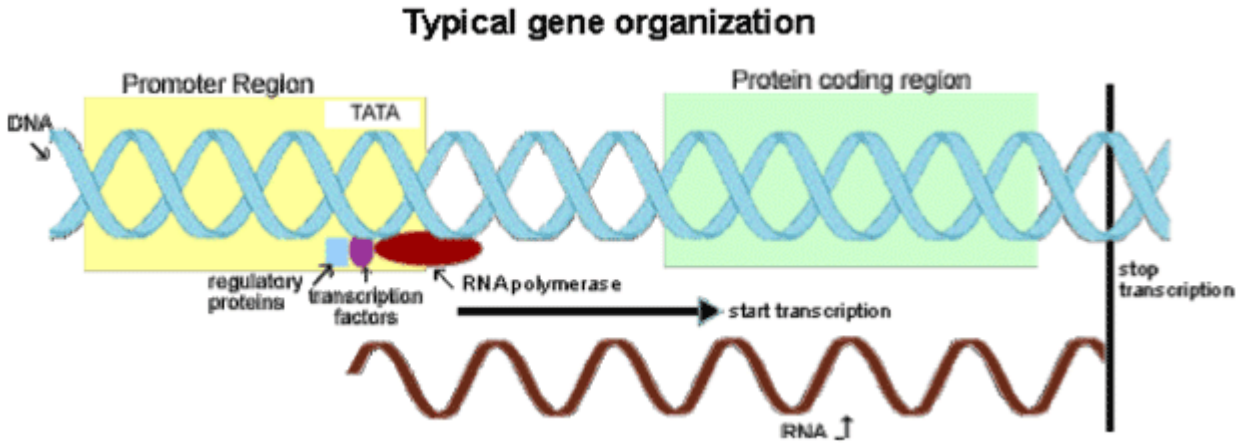


Diagram from Carolina Roa-Rodriguez

Nrf2 Activators Continued

- Proteins binding to promoter regions are known as **transcription factors**
- K. Itoh and colleagues of Tohoku University, Japan who identified Nrf2 as the main protein binding to AREs, thereby promoting phase 2 enzyme gene expression. Nrf2 is a transcription factor
- Paul Talalay and colleagues of Johns Hopkins University who helped to unravel how Nrf2 was activated by oxidants

- The above are only some of the people involved in the Nrf2 story

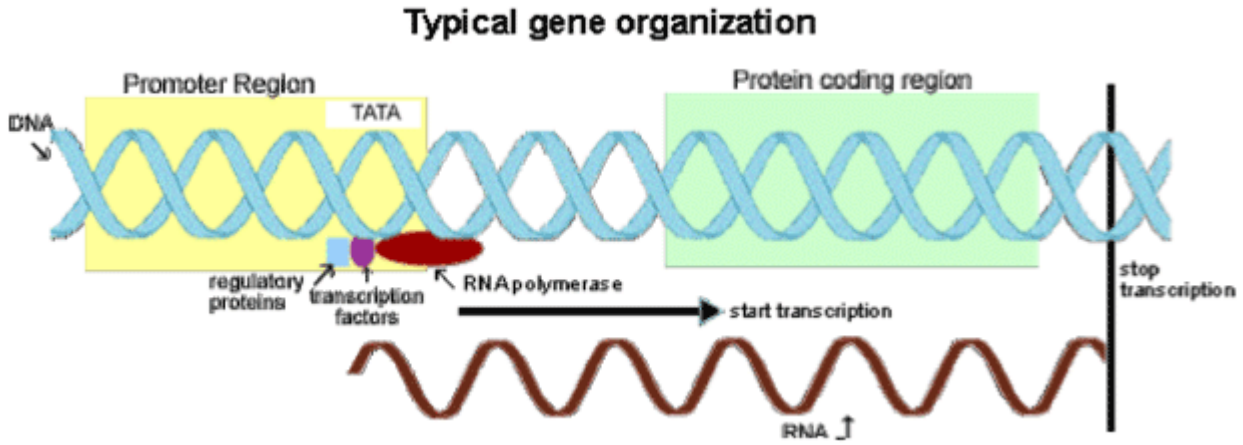
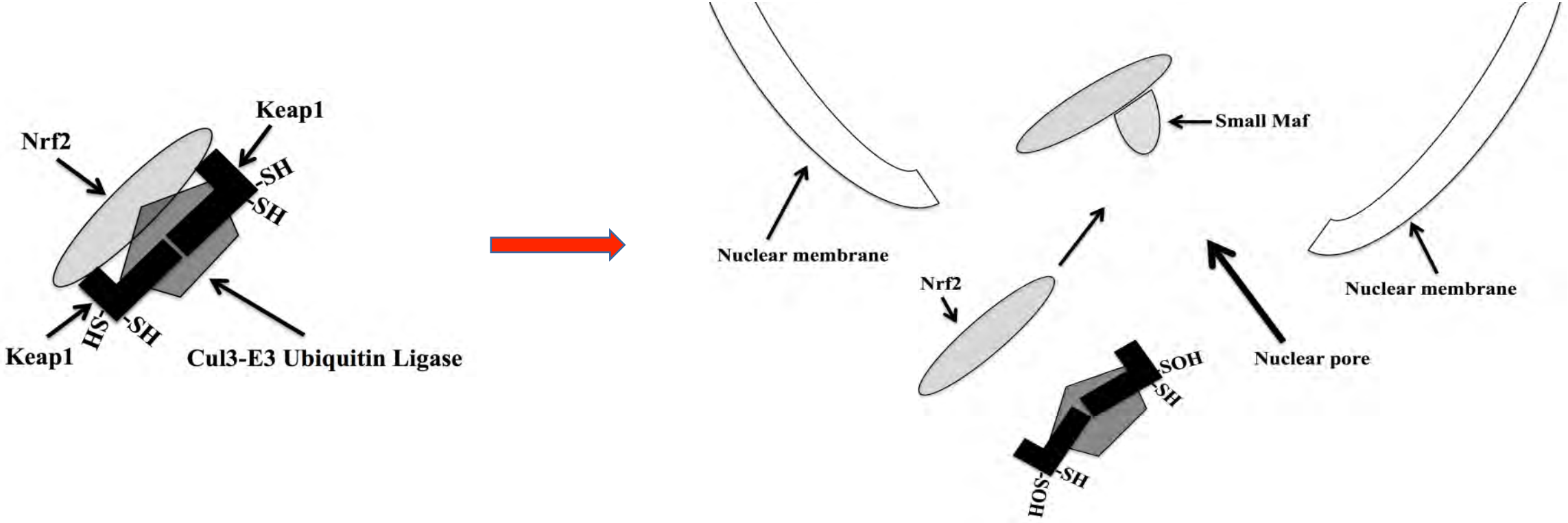


Diagram from Carolina Roa-Rodriguez

Nrf2 Signalling



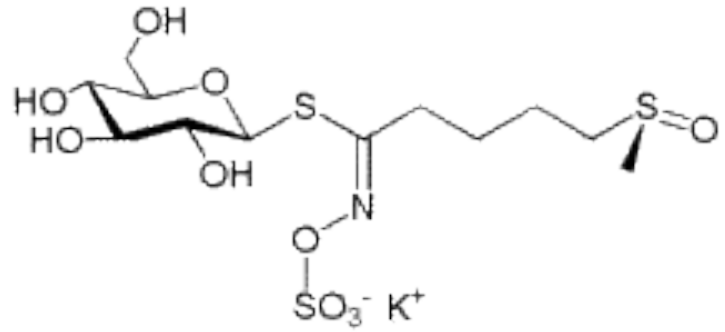
Diagrams taken from Juurlink. 2015. In Juurlink (Ed) **Broccoli: Cultivation, Nutritional Properties and Effects on Health**, Nova Publishers, New York, pp 111-119

Nrf2 activators oxidize Keap1 thiols that in turn allows Nrf2 to move into the nucleus and bind to AREs, thereby increasing the expression of dozens of genes whose protein products decrease oxidative stress and promote mitochondrial function. **Keap1 is a redox-sensing mechanism**

The Search For Dietary Nrf2 Activators

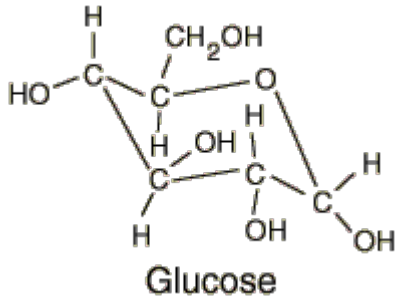
- Driven initially mainly by Paul Talalay and colleagues
- Discovered that compounds found within broccoli were potent Nrf2 activators
- Discovered that broccoli sprouts of certain cultivars had very high concentrations of Nrf2 activators
- Identified that a potent Nrf2 activator was sulforaphane, an isothiocyanate derivative of the glucosinolate, glucoraphanin (also known as sulforaphane glucosinolate)

Glucoraphanin Is Metabolized To Sulforaphane



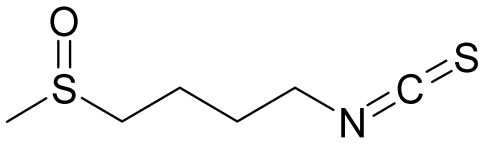
Glucoraphanin

Myrosinase



Glucose

+



Sulforaphane

- Johns Hopkins University obtained a patent in 1999 on broccoli sprouts – this was eventually overturned in 2001
- Paul Talalay and son licensed the patent and established Brassica Protection Products

Nrf2 Activators & Health

- The Talalay lab was initially interested in dietary Nrf2 activators for cancer prevention –following a path established by Lee Wattenberg
- I realized that Nrf2 activators not only would decrease the probability of cancer but would also result in lower oxidative stress and associated inflammation, topics that my lab were investigating
  - To provide arguments that this area was worthy of research funding I put together the evidence in a peer-reviewed paper:
    - Juurlink, B.H.J. 2001. Therapeutic potential of dietary phase 2 enzyme inducers in ameliorating diseases that have an underlying inflammatory component. *Canadian Journal of Physiology & Pharmacology* **79:** 266-282.

The Saskatchewan Agricultural Development Fund Provided Initial Research Funding

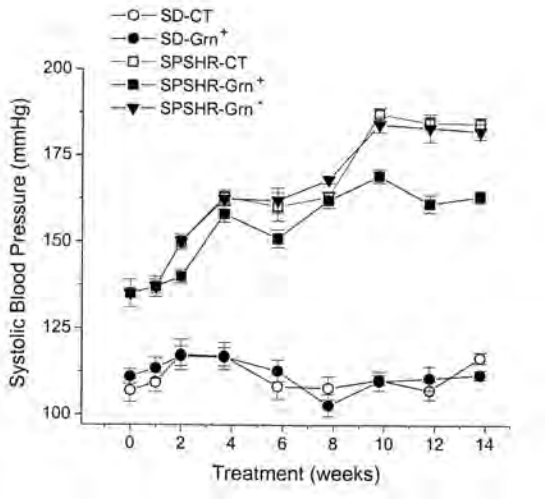
- It was difficult to get research funding since this is an area that was not classical nutrition nor classical pharmacology; however, the Saskatchewan ADF took a chance and gave my colleagues and I funding and I am forever grateful for this funding

- My colleagues and I started a series of experiments examining the ability of Nrf2 activators to decrease oxidative stress & associated inflammation in the Spontaneously Hypertensive Stroke-Prone (SHRsp) rat model of cardiovascular disease and we used the Sprague Dawley (SD) rat as the healthy rat model

- Jim and Maggie Mumm of Mumm's Sprouting Seeds, Parkside, Saskatchewan donated a huge quantity of Calabrese broccoli seeds for our research

Spontaneously Hypertensive Stroke-Prone (SHRsp) Rats Were Fed Broccoli Sprouts Daily

- Male animals divided into 3 groups:
  - 1) Control rat chow diet
  - 2) Rat chow plus dried Calabrese broccoli sprouts (200 mg daily) containing intact glucosinolates (5.5 micromoles sulforaphane glucosinolate)
  - 3) Rat chow plus dried Calabrese broccoli sprouts (200 mg) where more than 90% of the glucosinolates had been destroyed using freeze-thaw cycles



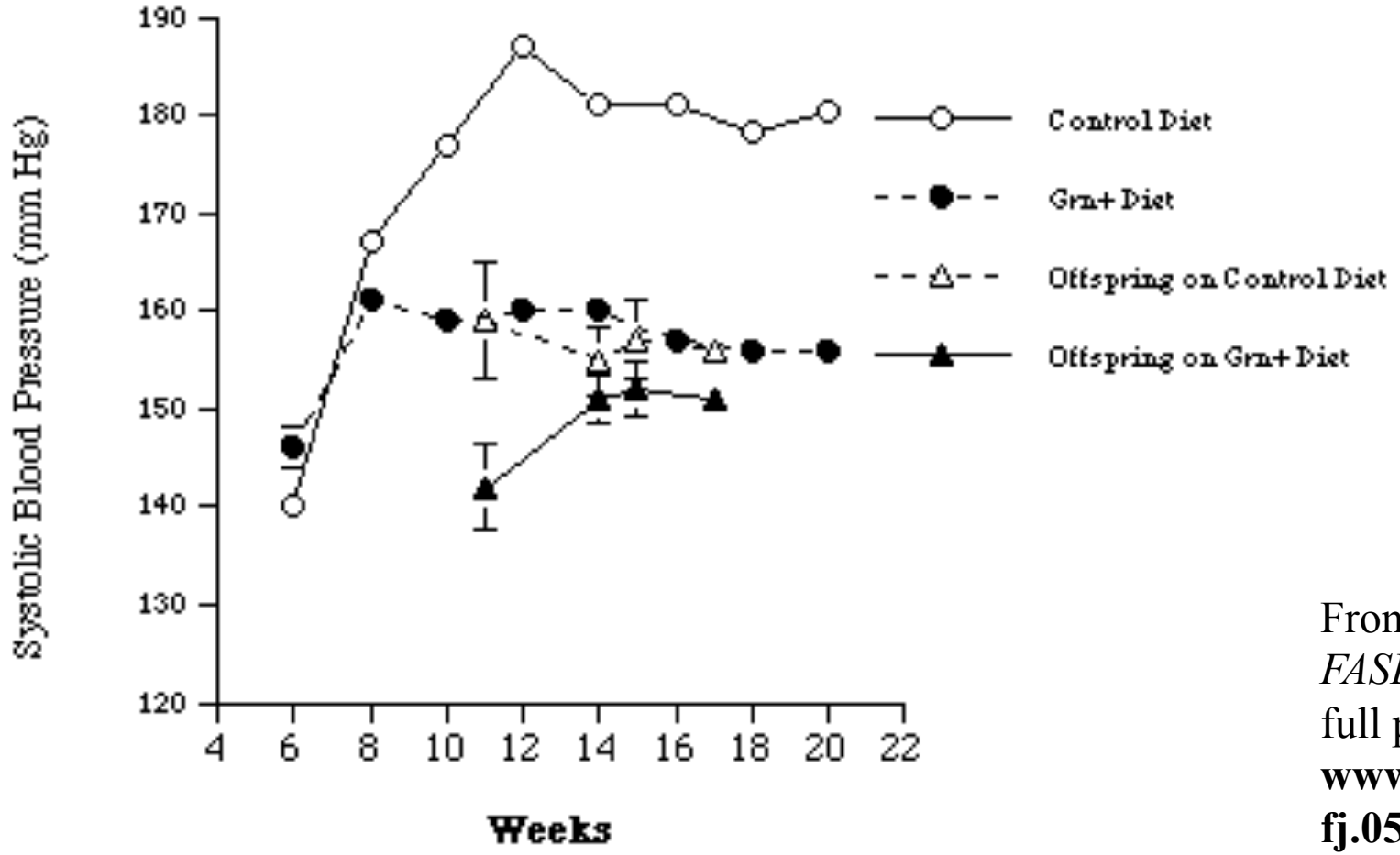
Summary of Dietary Outcomes In Male SHRsp

- Control and Depleted Glucosinolate diets led to high oxidative stress & inflammation as well as high blood pressures
- Diet supplemented broccoli sprouts with intact glucosinolates resulted in:
  - Decreased oxidative stress in all tissues examined
  - Increased oxidant-inactivation capacity in all tissues examined
  - Decreased inflammation in all tissues examined
  - Better endothelial function in arteries and lower blood pressure
  - For initial experimental results see:
    - Wu, L., M.H. Noyan Ashraf, M. Facci, R. Wang, P.G. Paterson, A. Ferrie and B.H.J. Juurlink. 2004. *Proceedings of the National Academy of Sciences* **101:** 794-799
    - Noyan-Ashraf *et al.* 2005. *Nutritional Neuroscience* **8:** 101-110
- There were no differences found amongst the SD treatment groups – suggesting that incorporating sulforaphane into the diet of healthy rats had no discernable physiological effect
- Will consider only a few aspects of additional experiments

Experiments With Female SHRsp

- Female rats were placed on control diet or diet supplemented with broccoli sprouts containing intact glucosinolates
- Some were mated with male rats on control diet and the offspring were placed either on control diet or on control diet supplemented with broccoli sprouts. Here the question that was being asked was whether decreasing oxidative stress during pregnancy can have positive effects on fetal determinants of adult health
- The results showed that the offspring of female SHRsp fed glucoraphanin-containing sprouts had significantly less oxidative stress & inflammation in all tissues examined and had lower blood pressures than their cousins whose mothers ate only control diet
  - These findings suggested that decreasing oxidative stress in the pregnant SHRsp has positive effects of fetal determinants of adult health

Effect of ± Glucoraphanin On Female SHRsp And Female Offspring Whose Mothers Were On Glucoraphin-Containing Diet

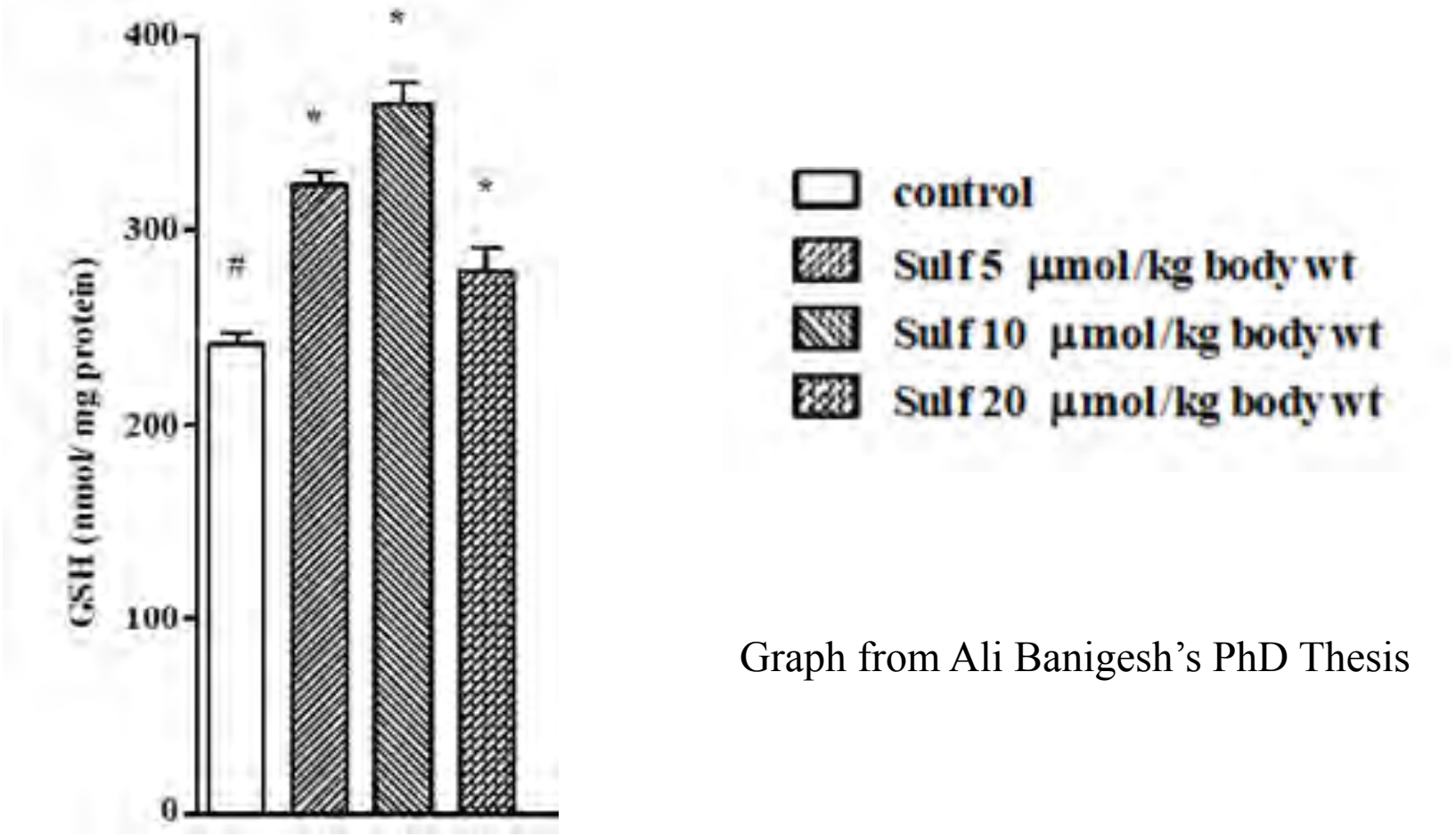


From: Noyan-Ashraf *et al.* 2006. *FASEB Journal* **20:** 371-373 with full paper online at: **http://www.fasebj.org/cgi/doi/10.1096/fj.05-4889fje**

Are These Effects Due To Sulforaphane?

- Since glucosinolate-depleted broccoli sprouts did not have positive effects on health we suspected that the positive health effects were due to sulforaphane but were not 100% sure since the freeze-thawing cycles may have introduced other changes in the sprouts
- To test this we administered either corn oil vehicle or sulforaphane in corn oil by gavage to female SHRsp rats for a period of 3 months and tested for oxidative stress, oxidant-inactivation ability and blood pressure
- Conclusion: The health-promoting effects seen when SHRsp consume broccoli sprouts with intact glucoraphanin is mainly due to glucoraphanin's metabolite, sulforaphane

Effect Of Sulforaphane On SHRsp Glutathione, A Major Player In Oxidant-Inactivation Mechanisms



Graph from Ali Banigesh's PhD Thesis

Fetal Determinants of Adult Health

- We know that adverse changes to the health of the mother increases the probability of developing type 2 diabetes, hypertension, obesity, etc
  - The starvation of half of the population of the Netherlands during the later part of World War Two gives human evidence of this
- Our studies were the first to show that diet can have a positive effect on fetal determinants of adult health
- Fetal determinants of adult health are due to changes in the regulation of gene expression during embryonic and fetal life
  - This area of study is known as **epigenetics** and what is studied is referred to as the **epigenome**

Does Decreasing Oxidative Stress & Inflammation Affect The Epigenome?

- The epigenome governs how readily genes are expressed.
- There are three major known mechanisms that regulate the epigenome:
  - **Methylation of deoxycytosines in DNA**
  - Modification of histone proteins
  - Micro-RNAs

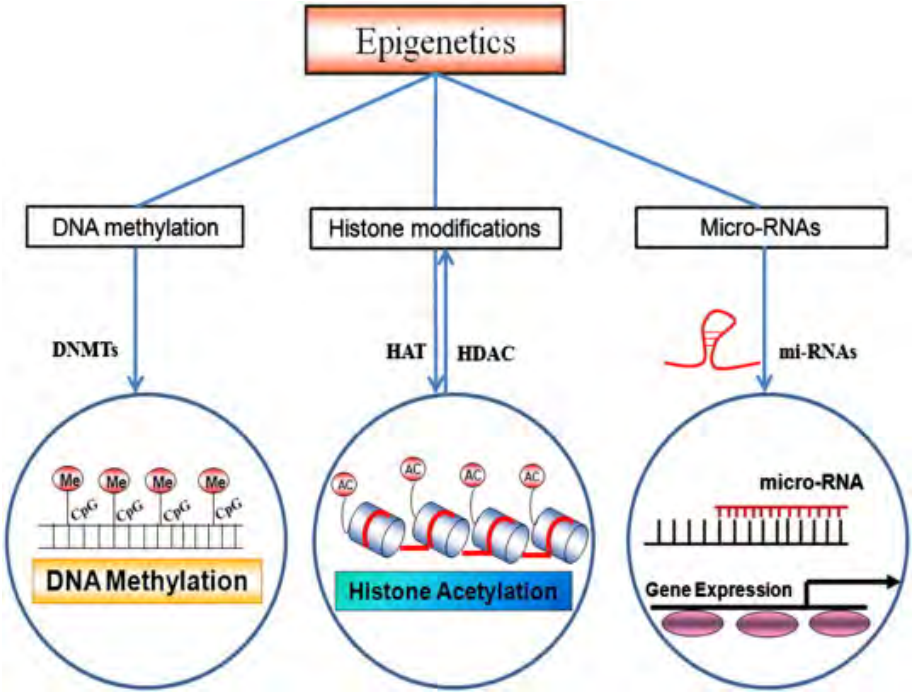


Image taken from P. Chaturvedi *et al.* 2014. *International Journal of Cardiology* **173:** 1-11

Kidneys Play A Major Role In Controlling Blood Pressure

- We examined global deoxycytosine methylation in the kidneys of SHRsp and SD rats gavaged with corn oil vehicle or sulforaphane in corn oil
- Sulforaphane normalized global renal deoxycytosine methylation

Table 2. Molar percentage of 5-methyl 2'-deoxycytosine to total 2'-deoxycytosine in kidney lysates of vehicle-treated and sulforaphane-treated SHRSP and SD rats

SHRsp		SD	
Vehicle	Sulforaphane	Vehicle	Sulforaphane
2.02 ± 0.03	2.31 ± 0.02*	2.28 ± 0.01	2.27 ± 0.01

* P<0.01 compared to vehicle SHRsp

Taken from Senanayake *et al.* 2012. *American Journal of Hypertension* **25:** 229-235

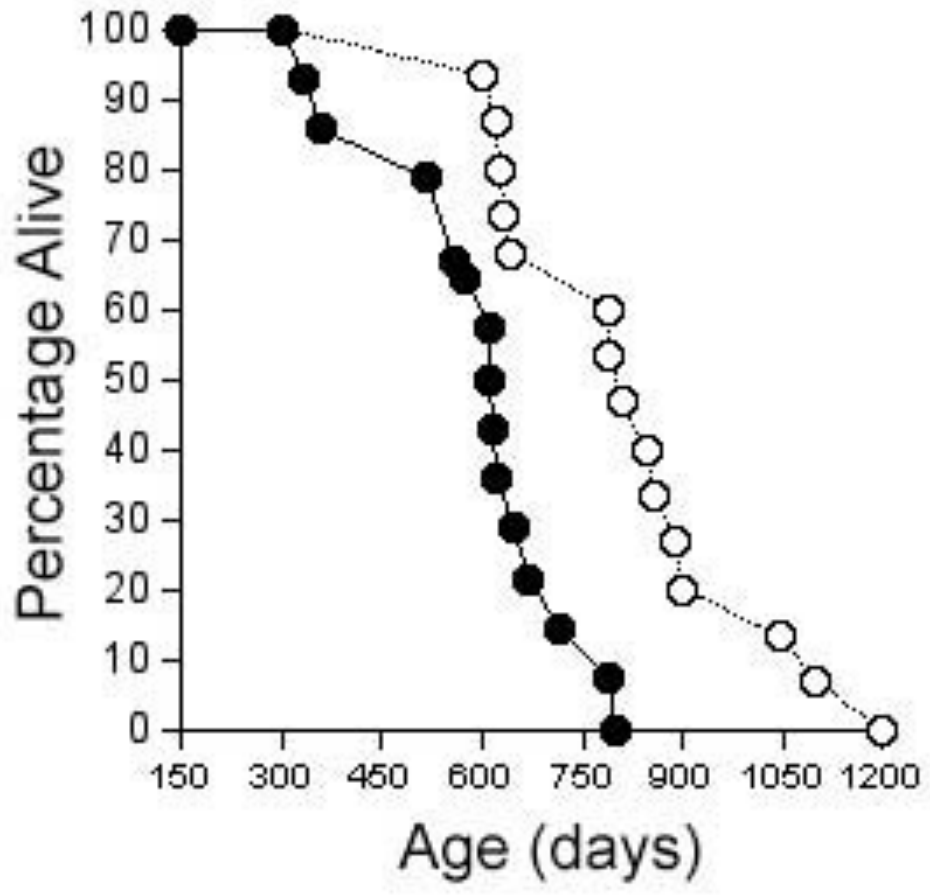
Nrf2 Activators

- Have positive effects on the epigenome in rats that are under oxidative stress
- Have no discernable effects on the epigenome of rats that have a normal healthy physiology

Does Consumption Of Nrf2 Activators Result In Healthier Aging?

- We examined this in mice.
  - Mice would not eat our dried broccoli sprouts so we chose to use tertiary-butylated hydroxyanisole (tBHA), a synthetic compound that is metabolized to Nrf2 activators
  - Results: the mice had less oxistress & inflammation during aging, were physically more agile and lived longer due to the healthier aging
    - See: Noyan-Ashraf *et al.* 2008. Phase 2 protein inducers in the diet promote healthier aging. *Journal of Gerontology A: Biological Sciences & Medical Sciences* **63:** 1168-1176.

Effect Of tBHA On Lifespan Of Mice



Closed circles were on control diet while open circles had tBHA added to the diet

From: Noyan *et al.* 2015. In Juurlink (Editor) **Broccoli: Cultivation, Nutritional Properties and Effects On Healt**h. Nova Publishers, New York, pp87-110

What About Human Health?

- A number of human clinical trials have been carried out, some of which have shown promise that high glucoraphanin-containing broccoli sprouts promote health
- Sprout ingestion containing up to 800 micromoles sulforaphane-equivalents daily by human trial participants had no discernable negative effects on physiological parameters
  - Thus, ingestion of high glucoraphanin-containing broccoli sprout that are low in goitrogenic glucosinolates is safe
  - **Nota bene: certain cultivars of broccoli have high concentrations of goitrogenic glucosinolates that interfere with thyroid function**

Effects On LDL-Cholesterol

- Several studies have shown that consuming high glucoraphanin-containing broccoli decreases the level of LDL cholesterol

  - Murashima *et al*. 2004. *Biofactors* **22:** 271-275
  - Armah *et al*. 2015. *Molecular Nutrition & Food Research* **59:** 918-926

  - These effects may be mediated by the effects of Nrf2 activators on mitochondrial function

Effects On Fasting Glucose & Insulin Resistance In Type 2 Diabetics

- Type 2 diabetics consumed either 0, 112 or 225 micromoles sulforaphane-equivalents in a broccoli sprout extract daily for a period of 4 weeks with 63 patients completing the study
- The 112 and 225 micromole sulforaphane-equivalents groups had significantly decreased fasting blood glucose levels: 1 and 2 millimole/L respectively
- The 225 micromole sulforaphane-equivalents groups had significantly lower blood insulin levels
- Bahadoran *et al.* 2012. *International Journal of Food Science & Nutrition* **63:** 767-771

- Note that in Type 2 diabetes there is oxidation of molecules critical in insulin signalling

Effect On Respiratory Passageways To Inactivate Air Pollutants

- High glucoraphanin broccoli sprout powder ingestion resulted in increased ability of nasal passageway cells to inactivate toxins
  - Riedl *et al.* 2009. *Clinical Immunology* **130:** 244-251
- Consumption of a broccoli sprout extract containing 100 micromoles sulforaphane-equivalent (4x daily) decreased allergic responses to diesel exhaust particles
  - Brandt *et al.* 2015. *Journal of Allergy & Clinical Immunology* **136:** 295-303
- Consumption of a broccoli sprout extract containing 800 micromoles glucoraphanin/sulforaphane increased inactivation diesel exhaust carcinogens such as benzene
  - Kensler *et al.* 2012. *Carcinogenesis* **33:** 101-107

Effect On Liver Function In Alcoholics

- Alcoholic patients with mildly impaired liver function consumed broccoli sprout extract (70 micromoles glucoraphanin daily) for two months
  - Kikuchi *et al.* 2015. *World Journal of Gastroenterology* **21:** 12457-12467

- Liver function improved
- Plasma oxistress decreased as determined by urinary 8-hydroxy-deoxyguanosine levels

Autism Spectrum Disorder

- Autism spectrum disorder is characterized by increased oxidative stress and mitochondrial dysfunction,
- Nrf2 activation decreases oxidative stress and improves mitochondrial function. This provided the rationale to treat autism through daily consumption of sulforaphane-rich broccoli sprout extracts (50 to 150 micromoles sulforaphane-equivalents, depending upon weight) for 22 weeks
- Consumption of broccoli sprout extracts significantly improved socially-impaired behaviours
  - Singh *et al.* 2014. *Proceedings of the National Academy of Sciences* **111:** 15550-15555
  - **The results of this study needs to be confirmed. Of interest is to see whether nutraceutical interventions early in childhood would have greater effects than later**

Schizophrenia

- Schizophrenia is also associated with increased oxidative stress, thereby providing a rationale for clinical trials using sulforaphane-enriched broccoli sprout extracts
- A small (7 patient) study demonstrated improved cognitive function in schizophrenics (ages ranging from 20 to 65 with a mean of 42.7 years) who consumed a broccoli sprout extract powder high in glucoraphanin/sulforaphane but no changes were seen in positive and negative aspects of the schizophrenia syndrome
  - Shiina *et al.* 2015. *Clinical Psychopharmacology & Neuroscience* **13:** 62-67
  - **This study needs to be repeated on a larger sample and it would be interesting to look at interventions in younger schizophrenics**

Human Clinical Trials Summary

- The human trials so far show great promise that consuming Glucoraphanin/Sulforaphane can ameliorate diseases that have an underlying oxistress and/or inflammation component(s)
- Consuming glucoraphanin/sulforaphane appears to tip the unhealth/ health balance to health

NUTRITION AND DIET RESEARCH PROGRESS

Broccoli

Cultivation, Nutritional Properties and Effects on Health

Bernhard H. J. Juurlink, Ph.D.
Editor

NOVA

Chapters:

- 1) Paul Talalay & Nrf2 activators
- 2) Glucosinolates & their distribution
- 3) Glucoraphanin/glucosinolates in broccoli
- 4) Drug/xenobiotic metabolism
- 5) Cellular redox: Aging & diet
- 6) The Nrf2 signalling system
- 7) Anti-inflammatory properties of quercetin and kaempferol
- 8) Protection potential of sulforaphane against radiation damage
- 9) Broccoli sprout supplementation as a novel approach to prevent perinatal brain injury

Chapters Continued:

- 10) Sulforaphane's potential to protect against UV light damage
- 11) Effects of sulforaphane on cardiovascular disease fetal determinants of adult health
- 12) Beneficial effects of broccoli sprouts & sulforaphane on type 2 diabetes
- 13) Human clinical studies involving sulforaphane/glucoraphanin
- 14) Broccoli cultivation in warmer climates
- 15) Agricultural characteristics & process harvest processing on glucosinolate content
- 16) Post-harvest conservation of broccoli
- 17) Innovative industrial cooking of broccoli for maintenance of health-promoting compounds

Nrf2 Activators Are Not A Panacea

- For example, clinical trials have no effect on chronic obstructive pulmonary disorder
  - Wise *et al.* 2016. *PLoS One* **11:** Article # e0163716
  - This is not surprising since COPD is associated with irreversible changes in lung structure
- If one has cancer it may not be desirable to activate the Nrf2 system, depending upon the cancer
- But Nrf2 activators should ameliorate all conditions associated with increased oxidative stress and/or mitochondrial dysfunction
- In order to make health claims we need larger clinical trials
  - Such Clinical Trials are expensive to fund and results in no Intellectual Property (IP)
  - Because of the lack of IP there is no incentive for Industry to run such trials
  - Pressure should be put on Public Institutions to fund such research

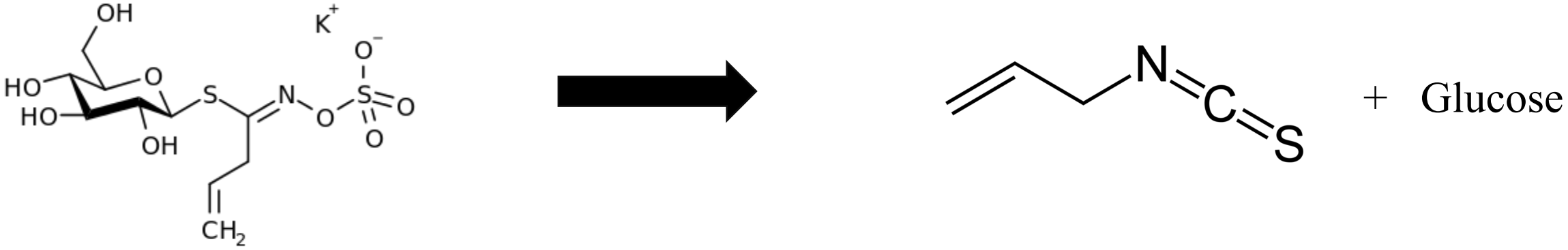
Brassica Cultivars High In Glucoraphanin And Low In Goitrogenic Glucosinolates

- We used Calabrese broccoli sprouts. It is likely that not all Calabrese lines have high glucoraphanin and low goitrogenic glucosinolates
- Hopkins cultivar – patented by USDA and developed by Dan Caudill, Mark Farnham & Greg Rieder (Caudill Seed)
- Beneforte broccoli: high glucoraphanin broccoli cultivar developed by Richard Mithen & colleagues at the John Innes Centre, Norwich, England
  - This cultivar, developed by breeding wild *Brassicas* from Southern Italy with broccoli, has been issued a patent and licensed to Monsanto
- Black Tuscany kale is also high in glucoraphanin and low in goitrogenic glucosinolates

There Are Other Glucosinolates That Activate The Nrf2 System

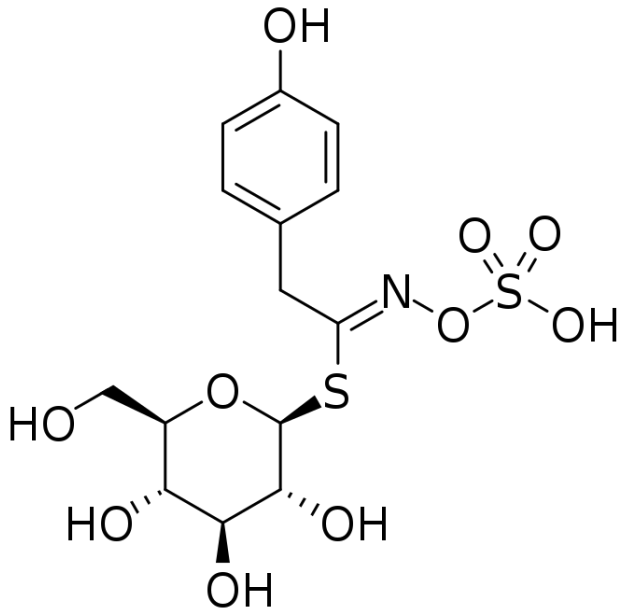
- For example, Sinigrin or allyl glucosinolate, gives rise to allyl isothiocyanate
  - Major glucosinolate in horseradish
  - Major glucosinolate in brown mustard (*Brassica juncea*) and black mustard (*Brassica nigra*)
    - *Brassica juncea* often eaten as mustard greens

- However, no clinical trials have been conducted with Sinigrin



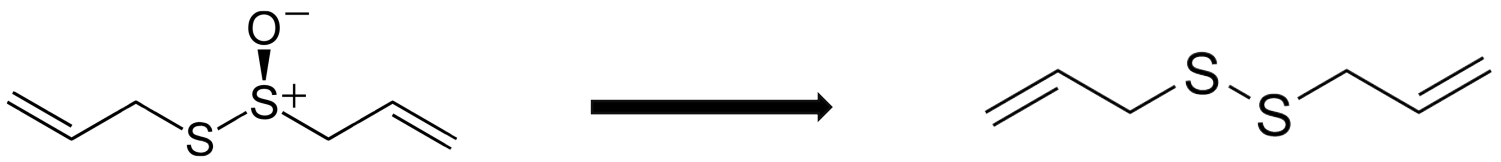
Beware Of Brassica With Goitrogenic Glucosinolates

- **For example, white mustard or *Sinapis alba* (*Brassica alba*) has levels of the goitrogenic glucosinolate sinalbin**



Some Examples Of Other Compounds That Have Nrf2-Activating Properties

- Diallyl disulfides, breakdown product of allicin
  - Gives garlic its characteristic odour
- Enterolactone, metabolite of the lignin SDG found in high levels in flax and sesame seeds
  - Also has estrogenic activities
- Protocatechuic acid (3,4-dihydroxybenzoic acid) – gut bacterial metabolite of flavonoids and anthocyanins

Allicin  Diallyl sulfide

Flavonoids Can Also Have Direct Therapeutic Effects

- Can aid in activation of Nrf2 by promoting phosphorylation of Nrf2
- Can inhibit non-infectious inflammatory pathways
- Flavonoids investigated for therapeutic properties include quercetin and kaempferol
  - High in crucifers and crucifer sprouts

Concluding Remarks

- There is clear evidence that dietary Nrf2 activators can have positive effects on health, particularly with chronic diseases that become more common with age
  - This should result in healthier aging
- We need more clinical trials with dietary Nrf2 activators and Functional Foods in general

What Can The ISGA Do?

- Can test for glucosinolate profiles of different crucifer lines for the members
- Can test for Nrf2 activators in non-crucifer lines of sprouts
- Advocate for more clinical trials in the area of Functional Foods