Engelhardt Institute of Molecular Biology
Russian Academy of Sciences

OXIDATIVE STRESS AND CARCINOGENESIS

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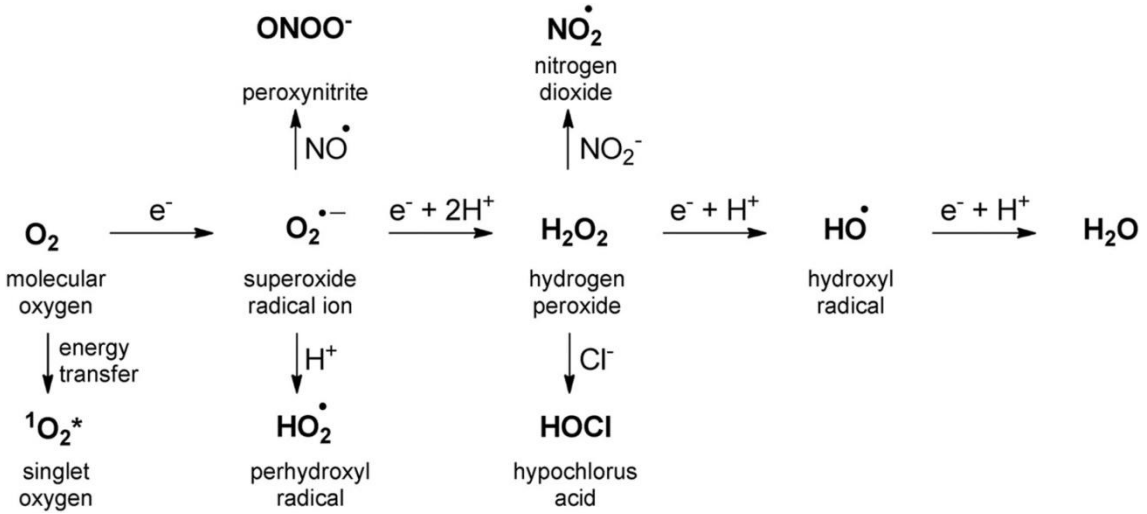
May 25, 2016

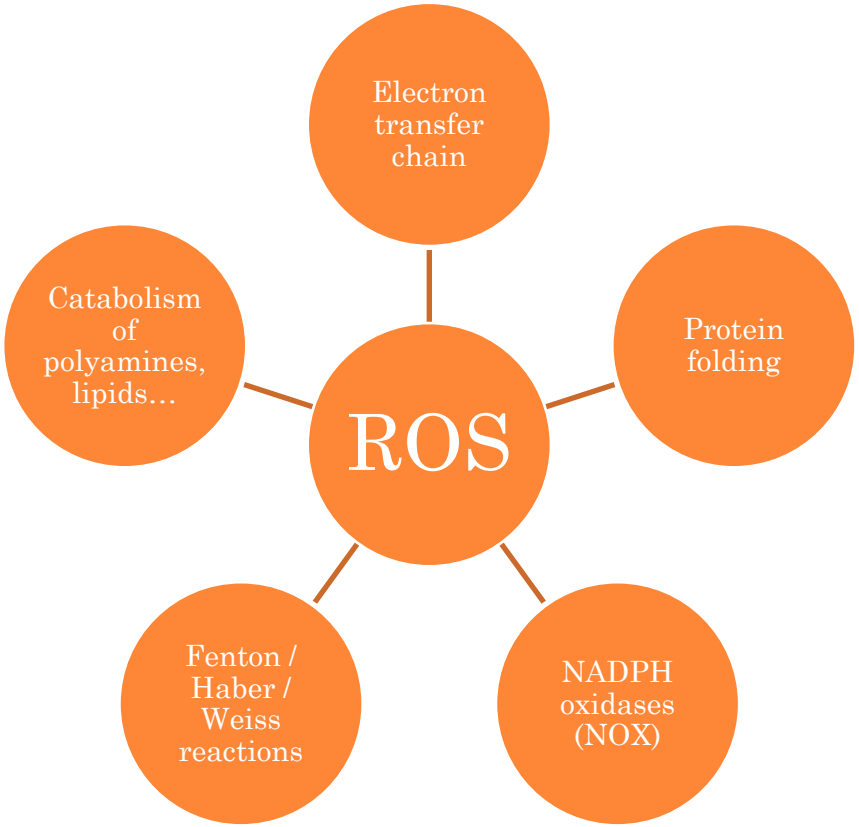
REACTIVE OXYGEN SPECIES

ROS		Half-life (s)	
$HO^{\bullet}$	Hydroxyl radical	10^{-9}	Highly reactive
$O_2^{\bullet -}$	Superoxide anion	10^{-6}	Poor oxidant
$^1O_2$	Singlet oxygen	10^{-6}	Highly reactive
H_2O_2	Hydrogen peroxide	10^{-3} - minutes	Poor oxidant



Adapted from *K. Krumova and G. Cosa (2016)*

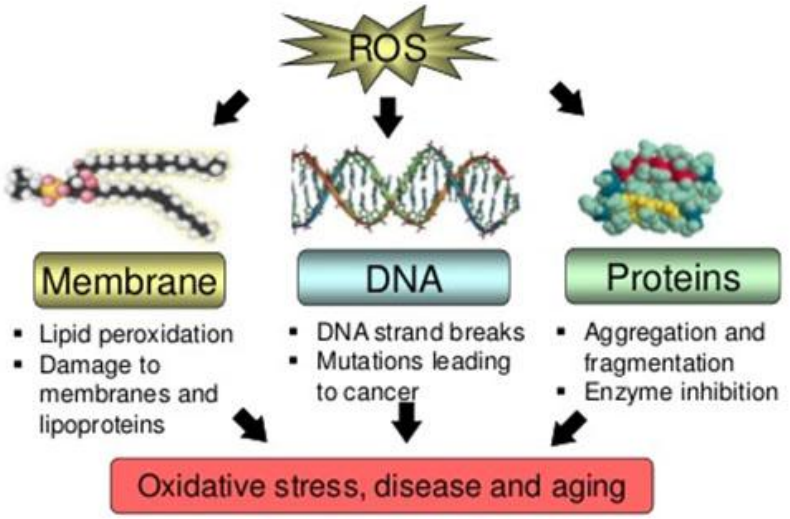
Reactive oxygen species (ROS)



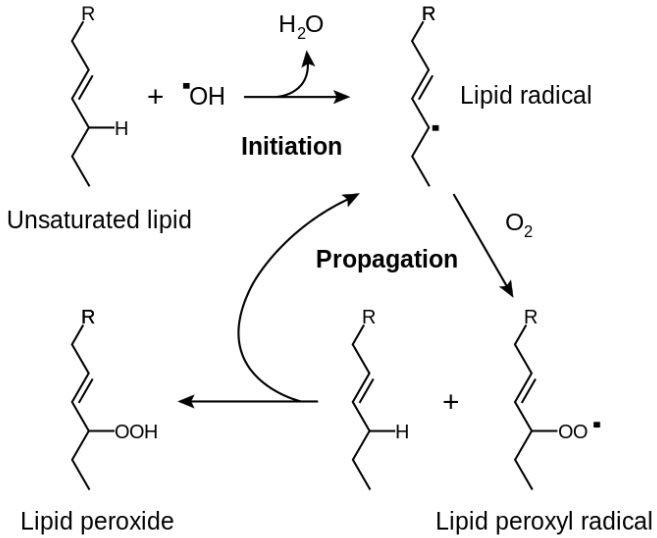
$$Fe^{2+} + H_2O_2 \xrightarrow{\text{Fenton Reaction}} Fe^{3+} + OH^{\bullet} + OH^{-}$$

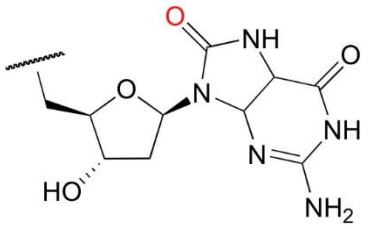
$$O_2^{\bullet-} + H_2O_2 \xrightarrow{\text{Haber-Weiss Reaction}} O_2 + OH^{\bullet} + OH^{-}$$

ROS DAMAGE DNA, LIPIDS, AND PROTEINS

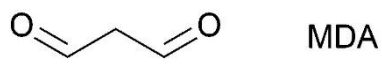


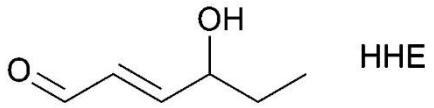
Adapted from *www.qiagen.com*

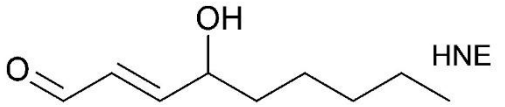




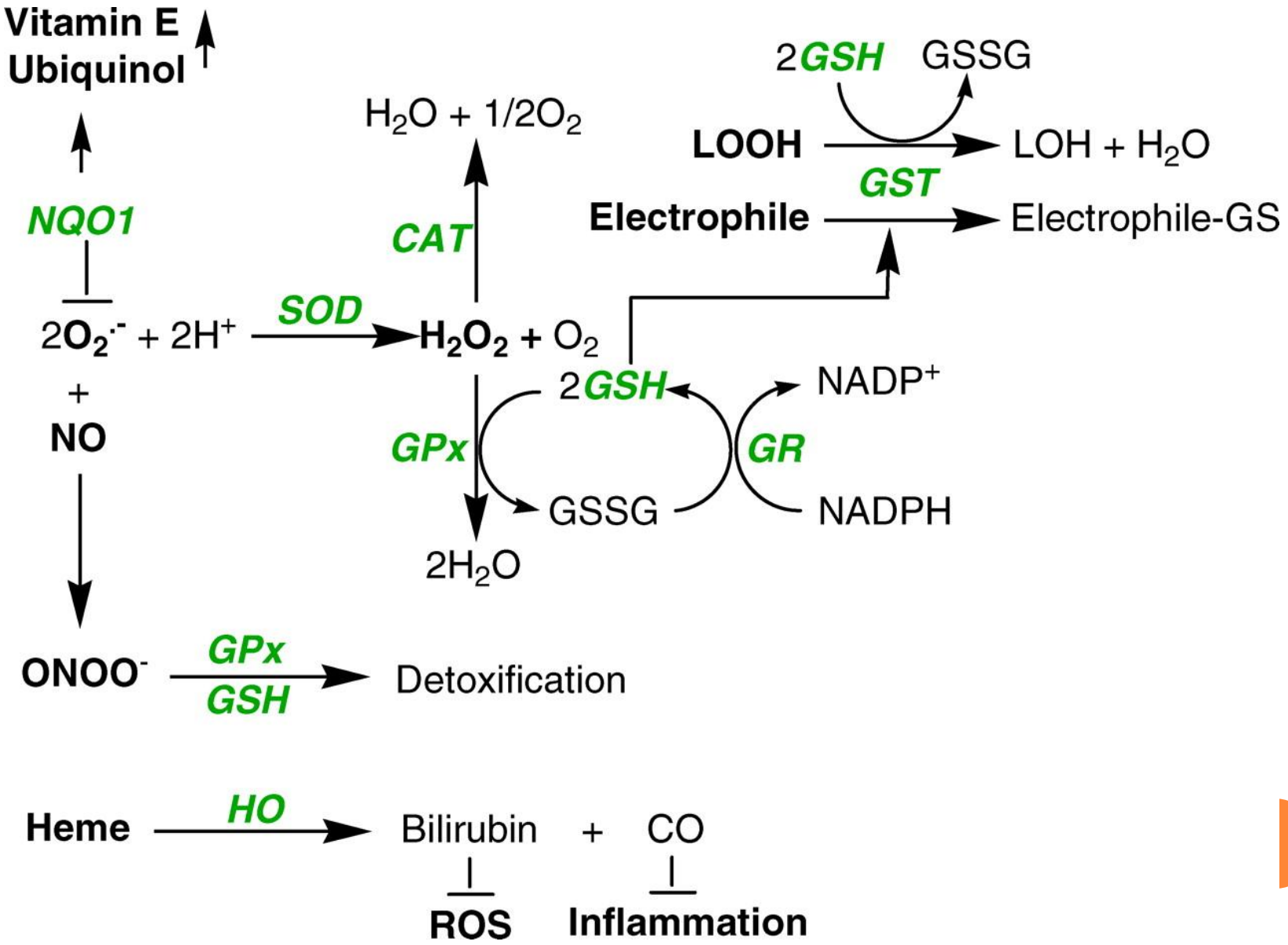
damaged 8-oxoguanine base







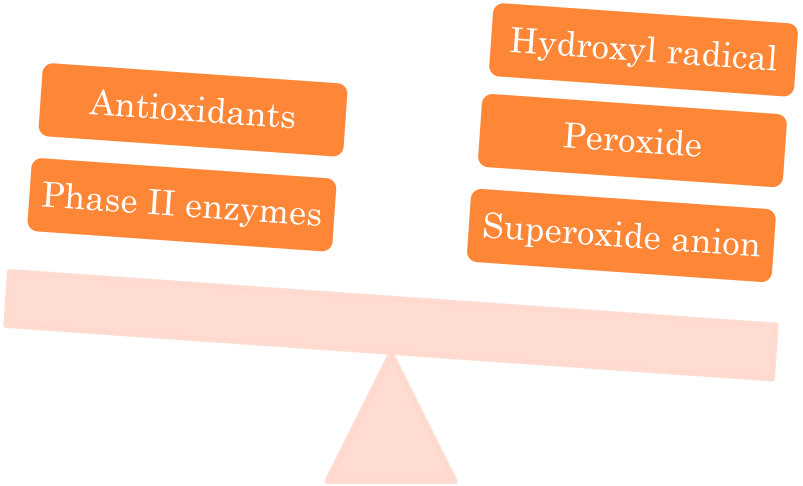
NEUTRALIZATION OF ROS



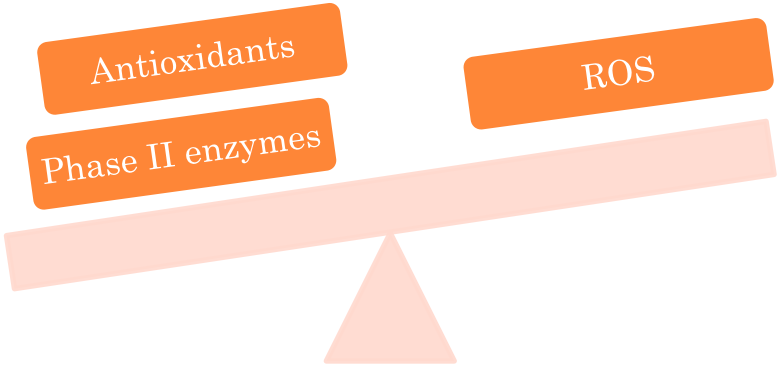
Adapted from *H.Zhu et al., Exp Biol Med 2008*

Oxidative stress

Oxidative stress is an imbalance between ROS production and antioxidant defenses



Prevalence of reducing agents over ROS is referred to as **reductive stress**



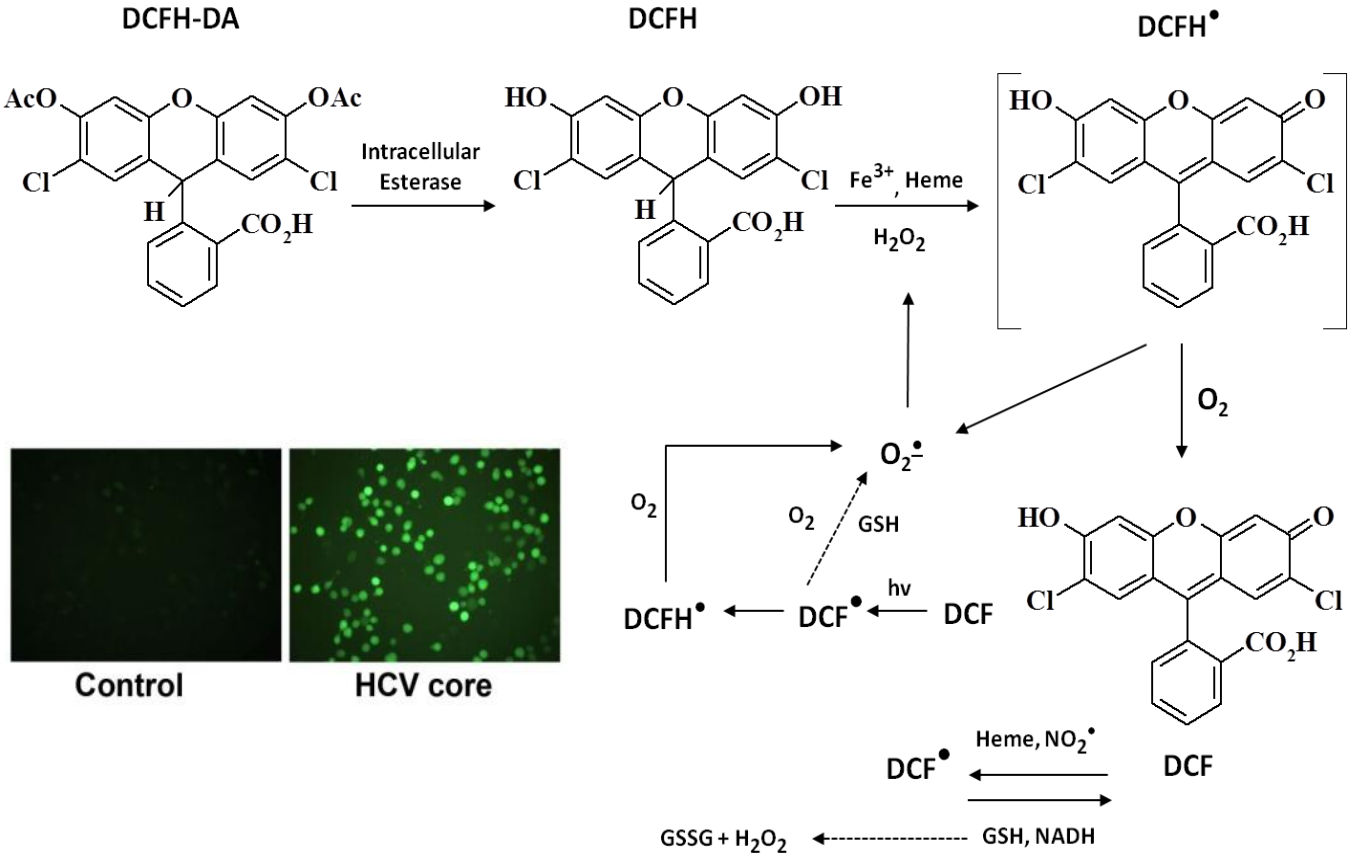
Detection of ROS and oxidation products

Direct ROS detection

Detection of oxidation products

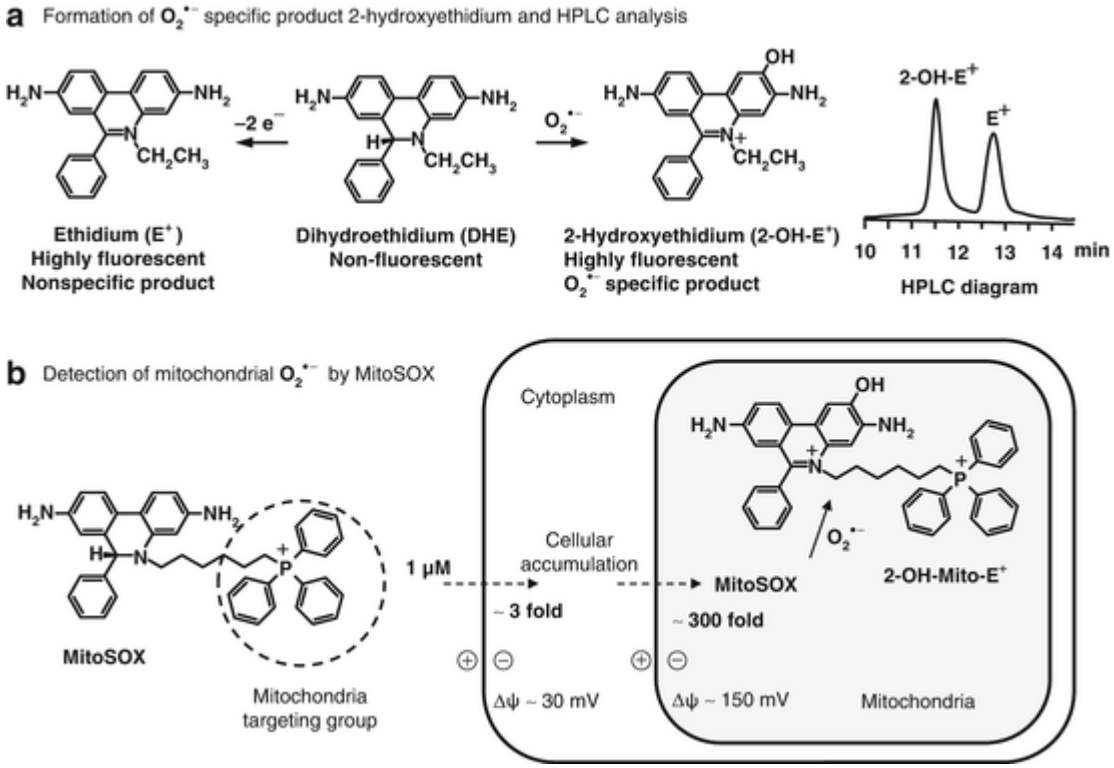
- **Proteins**
  - HS-groups
  - GSH/GSSG
  - 8-nitroTyr
    8-chloroTyr
  - Carbonylated proteins
- **Lipids**
  - Malondialdehyde (MDA)
  - 4-Hydroxy-nonenal (HNE)
  - F_2-Isoprostane
  - TBARS
- **DNA**
  - 8-oxoG
  - 5-hydroxyC
  - 8-nitroG

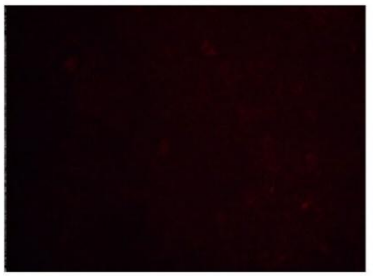
DETECTION OF ROS: $DCFH_2DA$



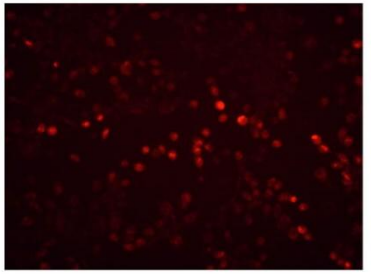
- $DCFH_2DA$ does not react with hydrogen peroxide. It reacts with hydroxyl-radical and other types of ROS/RNS. Thus it reflects general redox status
- $DCFH_2DA$ generates ROS

Detection of ROS: DHE and mitoSOX





Control

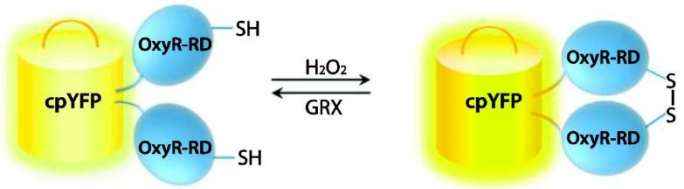


HCV core

S.*Dikalov & A.Dikalova, Systems Biology of Free Radicals and Antioxidants 2014*

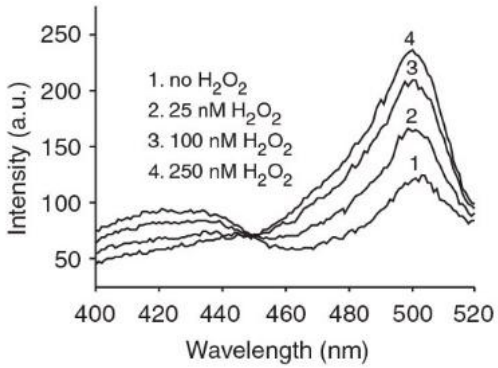
- DHE and MitoSOX react only with superoxide and no other type of ROS
- HPLC or mass-spec analysis is required for analysis of oxidation products

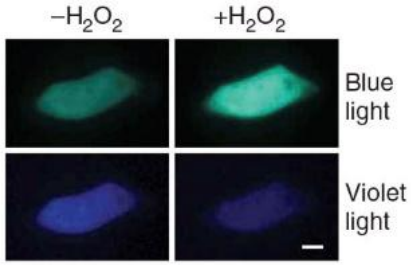
Detection of ROS: Ratiometric sensors

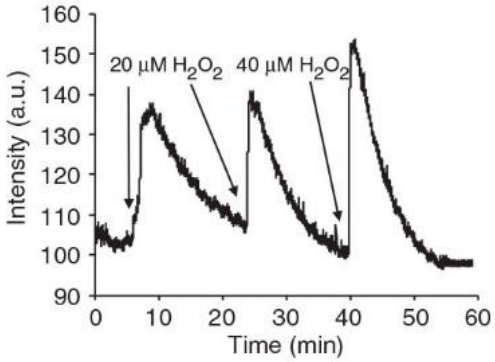


HyPER proteins

Adapted from *V.Belousov et al., Nat Protocols 2006, ARS 2016*

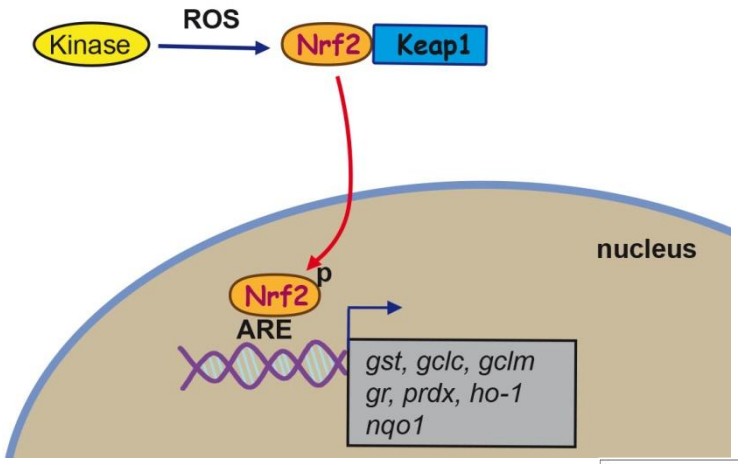






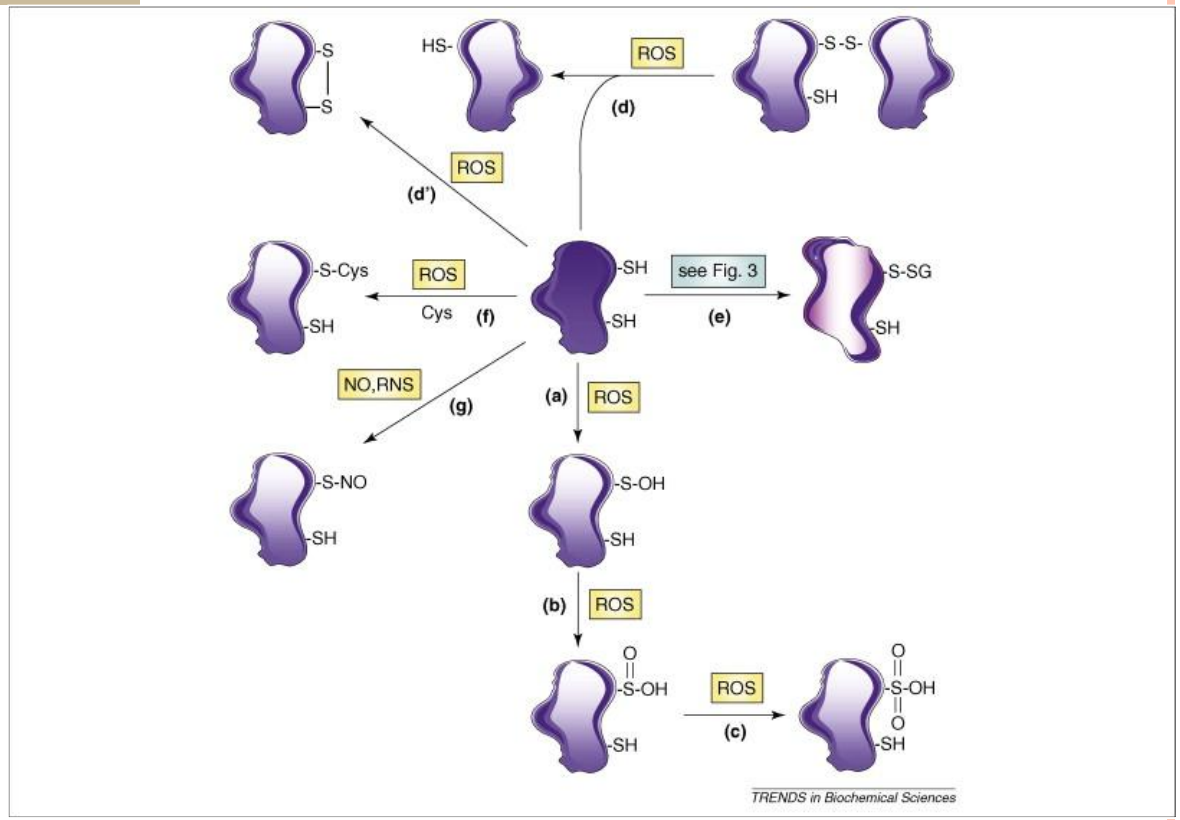
- HyPER proteins respond only to hydrogen peroxide
- Since plasmids are used, HyPER proteins allow measurement of H2O2 in cytoplasm, mitochondria, nucleus ets. The exception is endoplasmic reticulum
- Suitable for real-time measurements
- Drawback: pH-sensitive proteins. A control SyPER should be used.

NRF2/ARE PATHWAY

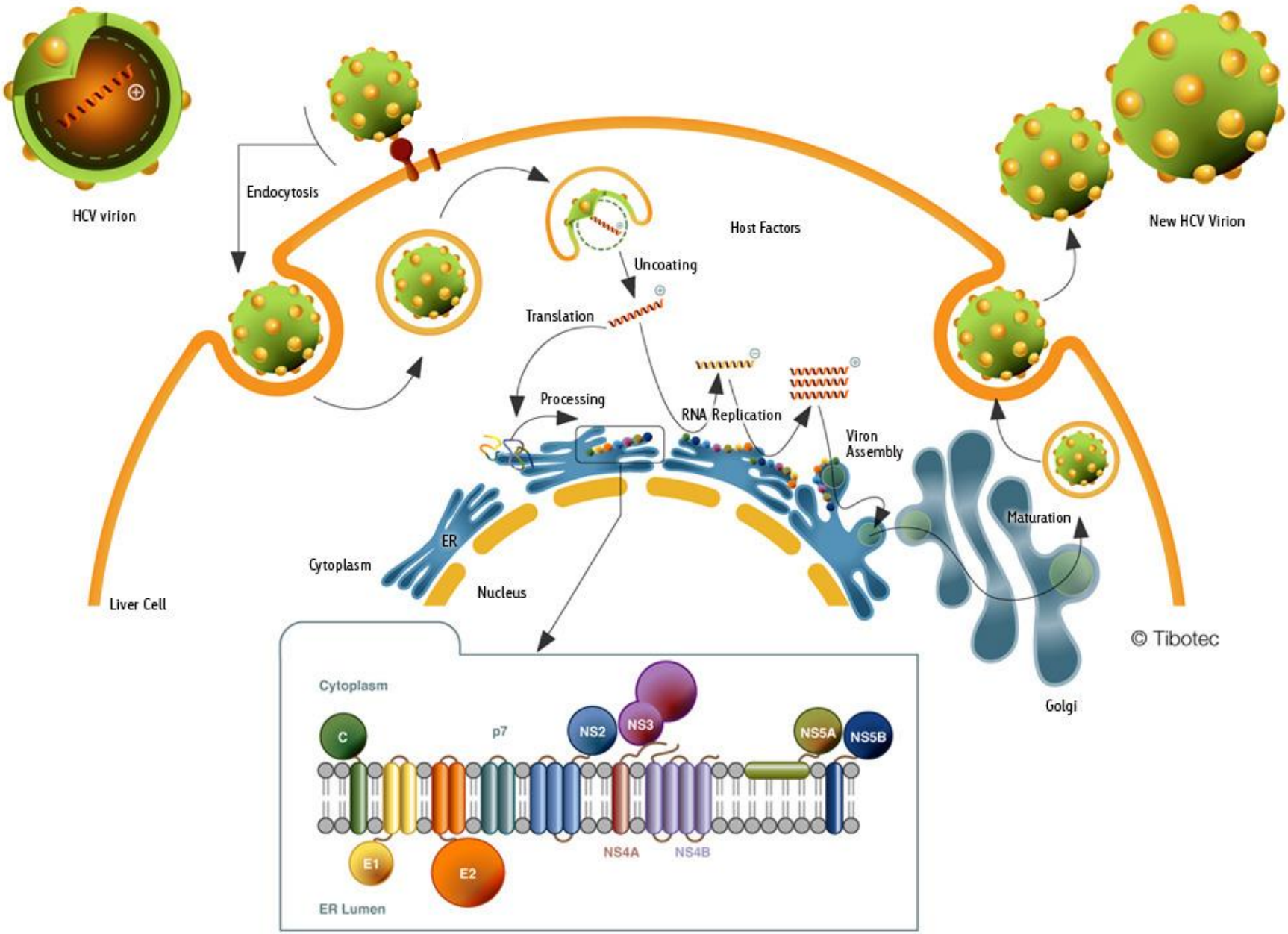


Nrf2/ARE pathway controls expression of genes encoding:
-Enzymes of glutathione biosynthesis
-Enzymes that scavenge ROS directly (Nqo1, Prdx etc)
-Heme oxygenase (HO-1)
-Phase III proteins (multidrug-resistant proteins etc)
-Metabolic enzymes

REDOX-SENSITIVE MODIFICATIONS OF PROTEINS



HCV LIFE CYCLE



REACTIVE OXYGEN SPECIES (ROS)

In the liver of chronic hepatitis C patients ROS levels are increased by 2-5 log, as estimated by EPR technique

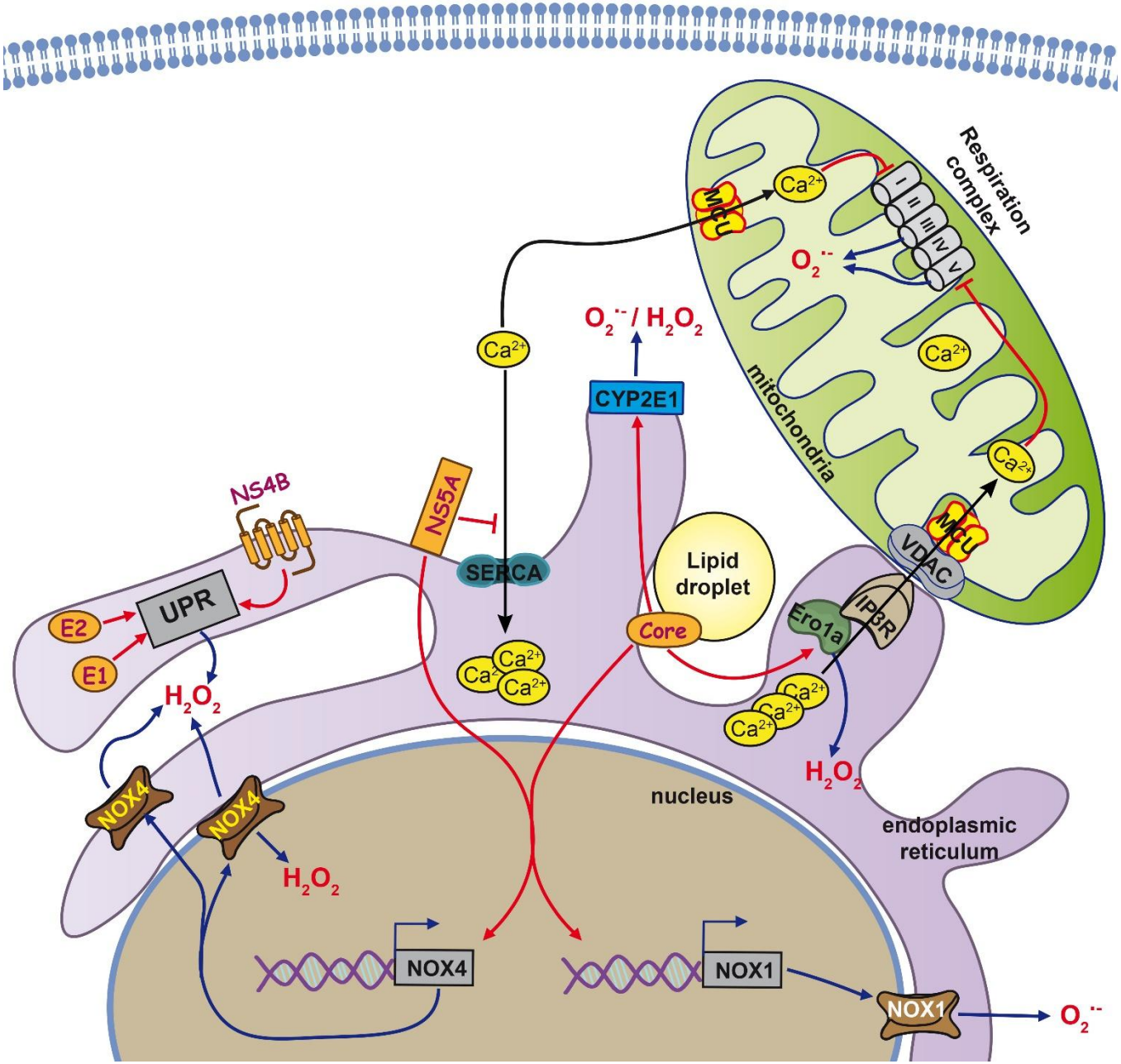
CHC patients exhibit elevated levels of oxidative stress markers and decrease antioxidant status…

	Control group (n:28)	CHC Patients (n:19) Before Treatment	*P*-value
MDA	4,20 ± 1,47	9,28 ± 1,61	<0.001
CuZn-SOD	285,78 ± 96,46	213,84 ± 71,61	<0.05
GSH-Px	8,01 ± 1,79	6,52 ± 1,86	<0.05
ALT	21,53 ± 6,02	95.84 ± 22.68	<0.001
AST	22,50 ± 4,91	80.52 ± 19.27	<0.001

…whereas elimination of infection reverts oxidative stress

	CHC Patients Before Treatment	CHC Patients After treatment	*P*-value
MDA	9,28 ± 1,61	4,88 ± 1,22	<0.001
CuZn-SOD	213,84 ± 71,61	357,94 ± 82,10	<0.001
GSH-Px	6,52 ± 1,86	9,47 ± 1,82	<0.001
ALT	95.84 ± 22.68	26,73 ± 10,65	<0.001
AST	80.52 ± 19.27	25,52 ± 8,68	<0.001

MECHANISMS OF ROS PRODUCTION IN HCV-INFECTED CELLS



Hepatitis B and C viruses promote liver cancer

Liver cancer is the 2d most common type of cancer (2012: app 745,000 cases – WHO)

Incidence of liver cancer increases by 2.3% per year (as was estimated for 2003-2012)

Therapy is ineffective, poor survival of patients

Hepatitis B and C viruses (HBV, HCV) account for 60-80% of all cases of hepatocellular carcinoma (HCC)

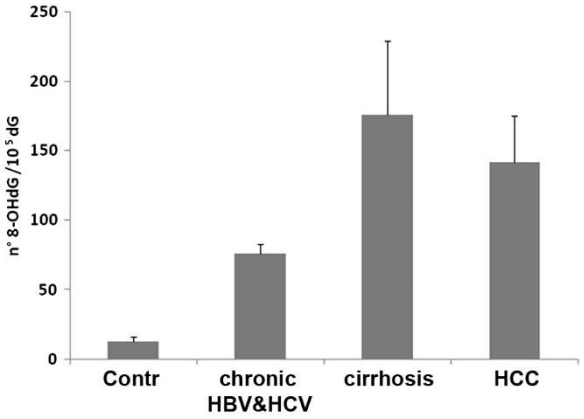
- Incidence rate in chronic hepatitis C patients – 1-5% per year
- Chronic hepatitis C increases HCC risk by 62.9 fold (Denmark)
- Elemination of infection reduces risk by 4.7 fold only

Genotypes of HCV affect rate of HCC incidence:

- Bruno et al (Hepatol, 2007): gt1 (4.26%) vs gt2a/b (1,69)
- Nikontchou et al (JVH, 2011): in pts with cirrhosis gt3 (34%) vs non-gt3 (17%)

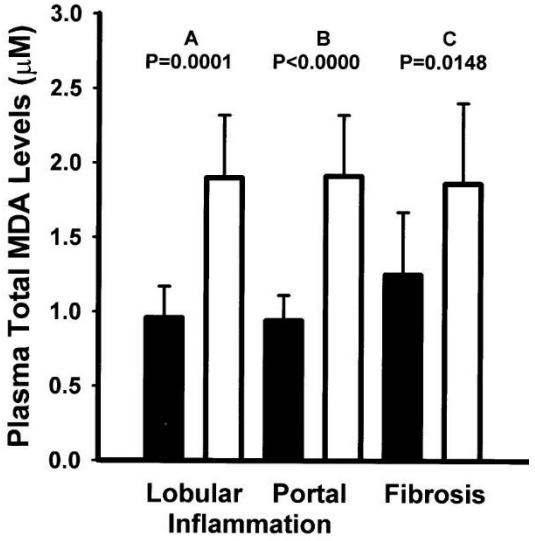
MARKERS OF OXIDATIVE STRESS CORRELATE WITH LIVER PATHOLOGIES

8-OxoG *vs* liver disease



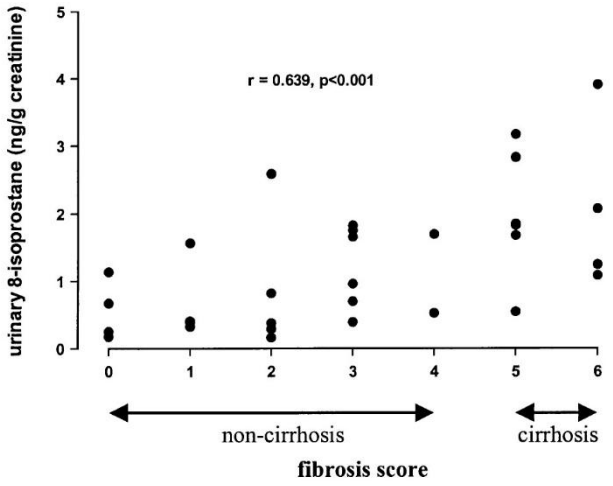
Adapted from *Cardin et al., BMC Cancer 2012*

Inflammation & tissue damage

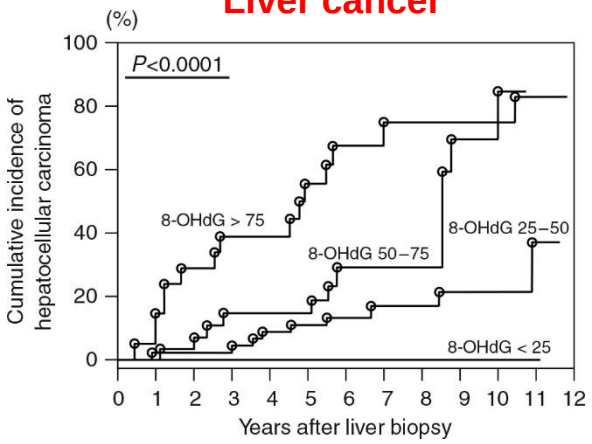


Yadav et al., Am J Gastro 2002

Fibrosis



Liver cancer



Tanaka et al., Br J Cancer 2008

HCV-ASSOCIATED LIVER CANCER IS ASSOCIATED WITH CHRONIC INFLAMMATION, ADVANCED FIBROSIS, AND OXIDATIVE STRESS,

Characteristics	HCC group ($N=36$)	Non-HCC group ($N=82$)	P-value
Age (years)	57.3 ± 8.2	54.7 ± 11.4	0.3718[a]
Laboratory data			
ALT ($IU\,l^{-1}$)	91.9 ± 50.4	65.6 ± 52.9	**0.0021**[a]
AST ($IU\,l^{-1}$)	91.4 ± 42.7	60.5 ± 38.3	**0.0003**[a]
Liver histology			
Inflammatory activity (0/1/2/3)[c]	0/4/18/14	1/37/31/13	**0.0015**[b]
Fibrosis staging (0/1/2/3/4)[d]	0/1/3/10/22	1/28/23/17/13	**<0.0001**[b]
Total iron score[e]	11.09 ± 4.75	6.23 ± 5.62	**<0.0001**[a]
8-OHdG-positive hepatocytes (per $10^5\ \mu m^2$)	65.2 ± 20.2	40.0 ± 23.5	**<0.0001**[a]

Tanaka et al., Br J Cancer 2008

[CANCER RESEARCH 61, 4365–4370, June 1, 2001]

Oxidative Stress in the Absence of Inflammation in a Mouse Model for Hepatitis C Virus-associated Hepatocarcinogenesis[1]

Kyoji Moriya, Kiyotaka Nakagawa, Tomofumi Santa, Yoshizumi Shintani, Hajime Fujie, Hideyuki Miyoshi, Takeya Tsutsumi, Teruo Miyazawa, Kotaro Ishibashi, Toshiharu Horie, Kazuhiro Imai, Toru Todoroki, Satoshi Kimura, and Kazuhiko Koike[2]

Department of Internal Medicine, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655 [K. M., Y. S., H. F., H. M., T. Ts., S. K., K. K.]; Biodynamic Chemistry Laboratory, Tohoku University Graduate School of Life Science and Agriculture, Sendai 980-8575 [K. N., T. M.]; Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo 113-0033 [T. S., K. Im.]; Daiichi Pharmaceuticals, Tokyo 134-0081 [K. Is.]; Department of Pharmacology, University of Chiba, Chiba 260-8670 [T. H.]; and Department of Laboratory Medicine, Keio University School of Medicine, Tokyo 160-8582 [T. To.], Japan

ABSTRACT

The mechanism of hepatocarcinogenesis in hepatitis C virus (HCV) infection is still undefined. One possibility is the involvement of oxidative stress, which can produce genetic mutations as well as gross chromosomal

hepatitis (3). If this is the case, HCV would only be indirectly associated with hepatocarcinogenesis. Another possibility is the direct involvement of HCV in hepatocarcinogenesis, whereby the product of the virus may be oncogenic and involved in cell transformation.

HCV CORE, NS3, AND NS5A PROTEINS PROMOTE CARCINOGENESIS

HCV transgene	*Genotype*	*Promoter*	*Genetic background*	*Pathology*	*Cancer frequency*	*Reference*
Polyprotein	1b	Albumin	C57BL/6	Steatosis, HCC	5/37	(Lerat *et al.*, 2002)
Core	1b	HBV	C57BL/6	Steatosis, HCC	14-31%	(Moriya *et al.*, 1998)
Core-E1-E2	1a	CMV	B6C3F1	Steatosis, variety of tumors of hepatic and non-hepatic origin	17/185	(Naas *et al.*, 2005)
NS5A	1b	HBV	C57BL/6J × CBA/J	Steatosis, tumors	10/163	(Wang *et al.*, 2009a)

Adapted from *McGivern and Lemon, Oncogene 2011*

ELSEVIER

Cell Biology International 30 (2006) 915–919

Cell Biology International

www.elsevier.com/locate/cellbi

Different transformation pathways of murine fibroblast NIH 3T3 cells by hepatitis C virus core and NS3 proteins

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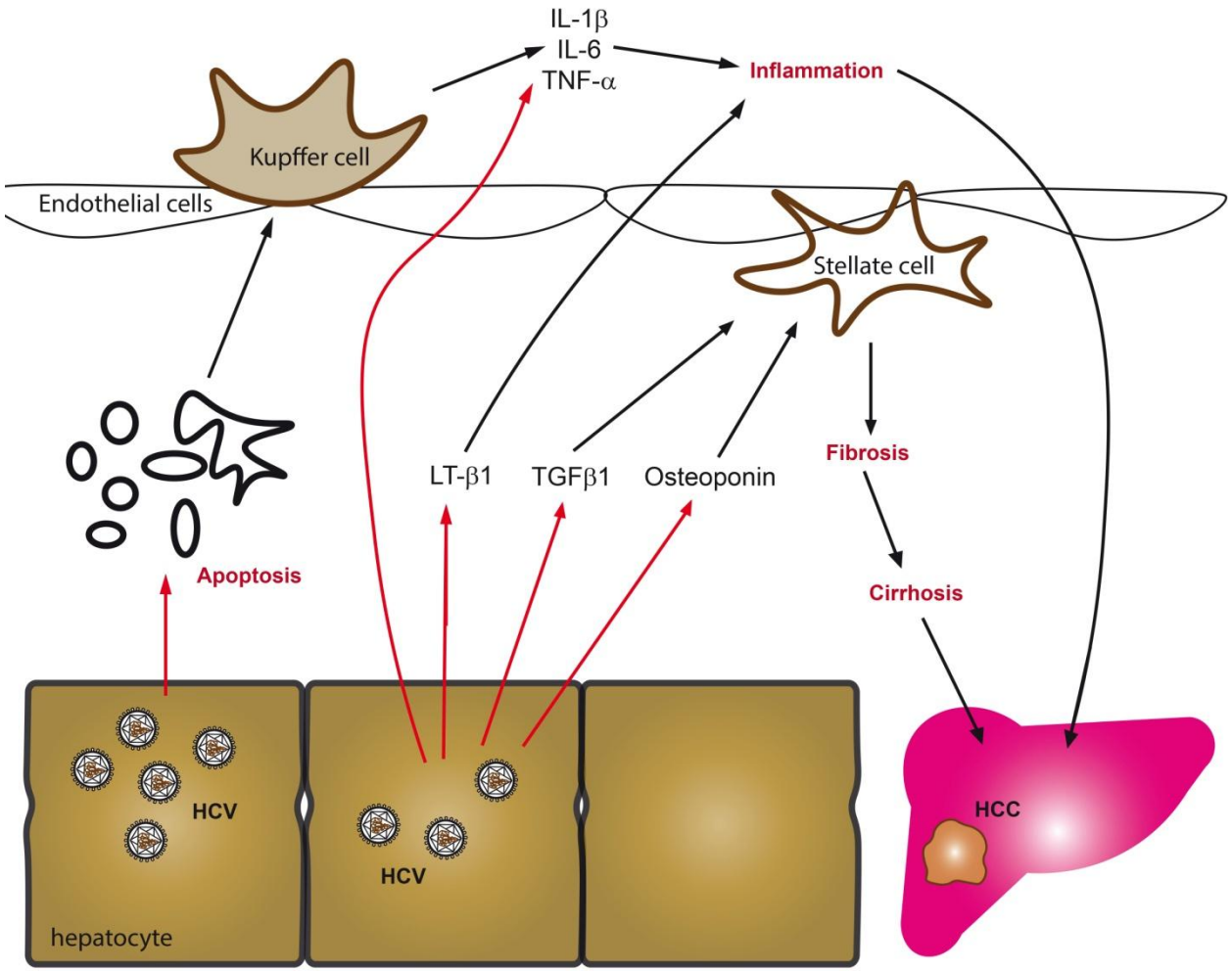
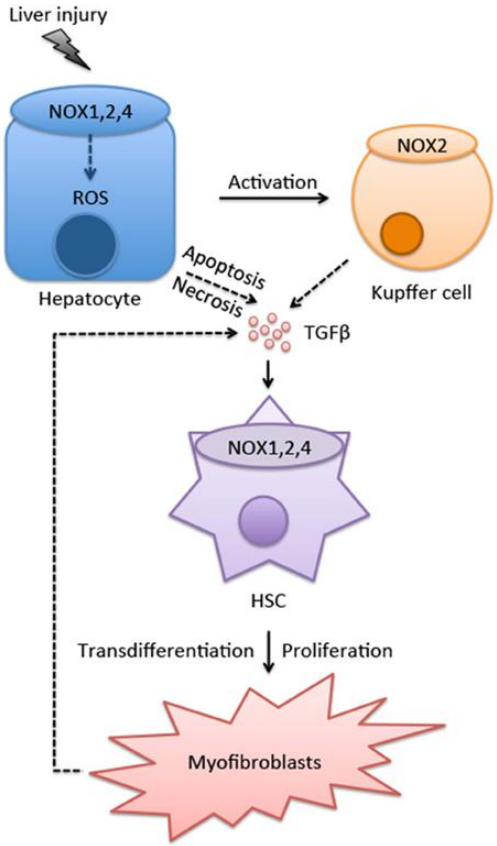
Received 25 April 2005; revised 14 April 2006; accepted 8 June 2006

Abstract

The oncogenic potential of both Hepatitis C virus (HCV) core and HCV NS3 proteins has been demonstrated, but these proteins induce transformation of immortal murine fibroblasts NIH 3T3 via different pathways. As long-term expression (50–100 passages) of HCV core triggers neoplastic transformation of NIH 3T3 through crisis of growth, HCV NS3 induces transformation shortly after transfection. We explain this distinction by different effects of core and NS3 on p53-mediated transactivation: inhibition by NS3 and activation by core protein.

CARCINOGENESIS IN THE LIVER



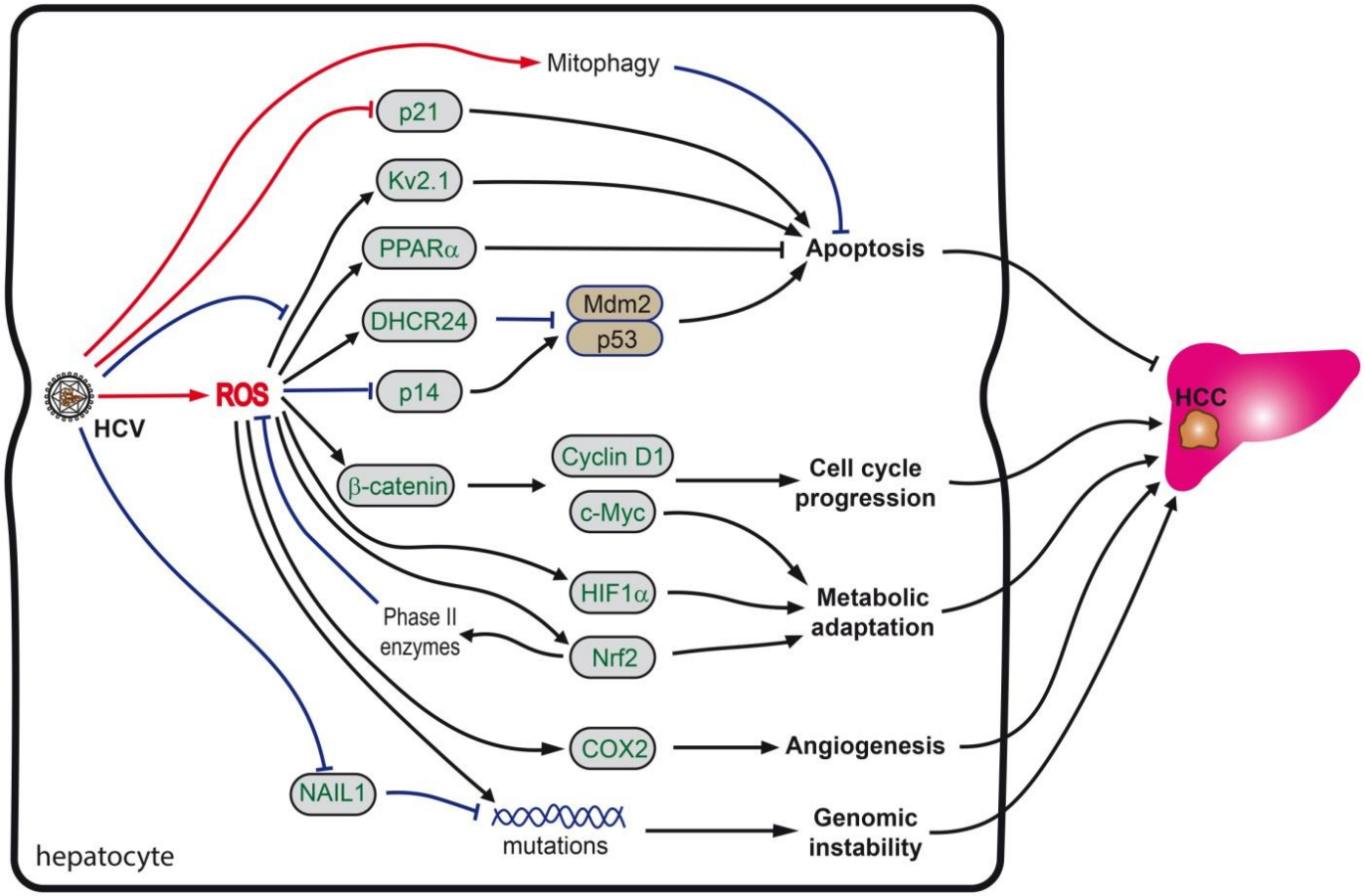


Liang et al., Fron Phisiol 2016

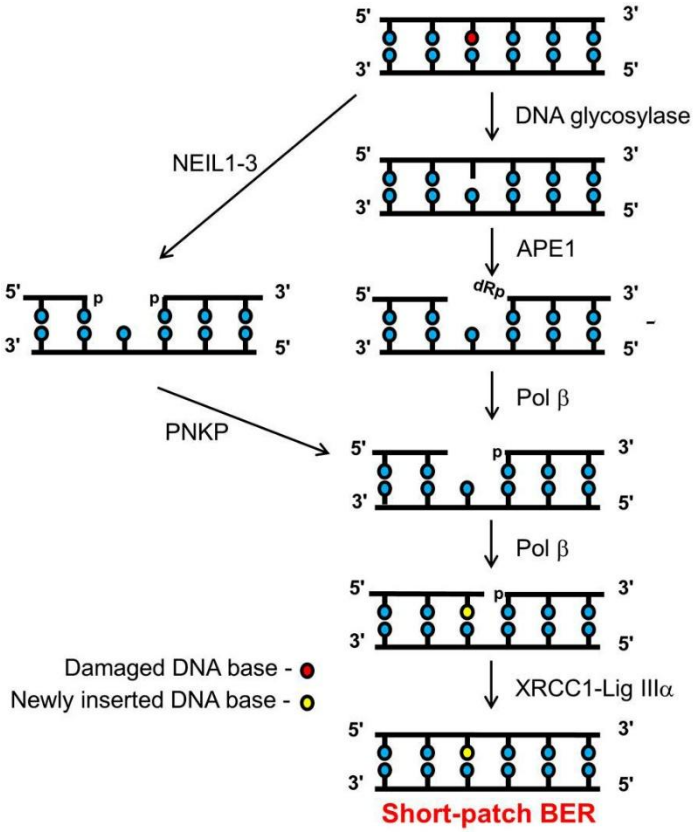
HCV-induced oxidative stress contributes to production of TGFb1 and proinflammatory cytokines in hepatocytes, may lead to cell death followed by production of cytokines by Kuppfer cells etc…

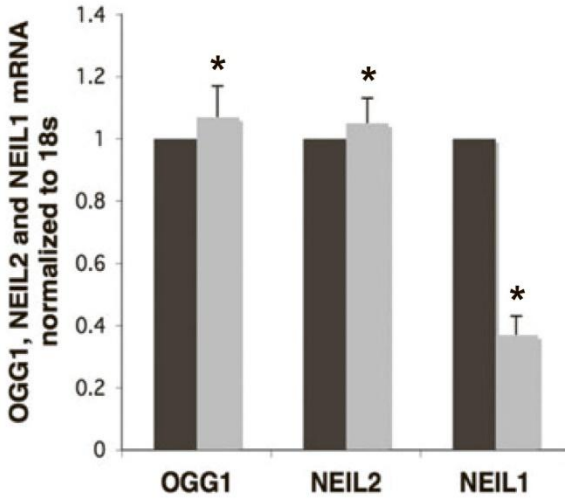
ROS produced by NADPH oxidases also play crucial ROS in activation of HSCs dring fibrogenesis.

Reactive oxygen species (ROS)



HCV INHIBITS REMOVAL OF 8-OXOG BY BASE-EXCISION REPARATION

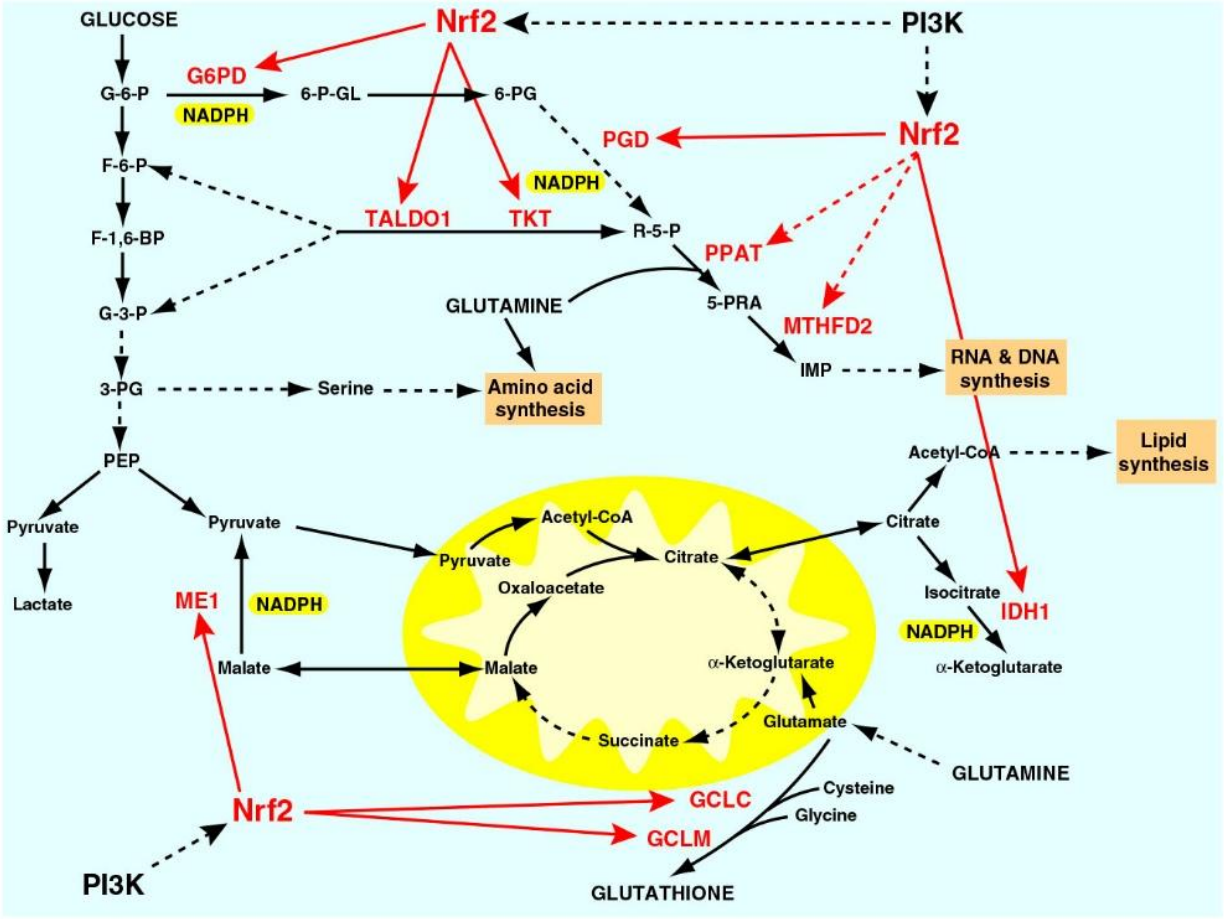




Pal et al., J Gastro Hepatol 2010

Adapted from *Nickson & Parsons, Front Genet 2014*

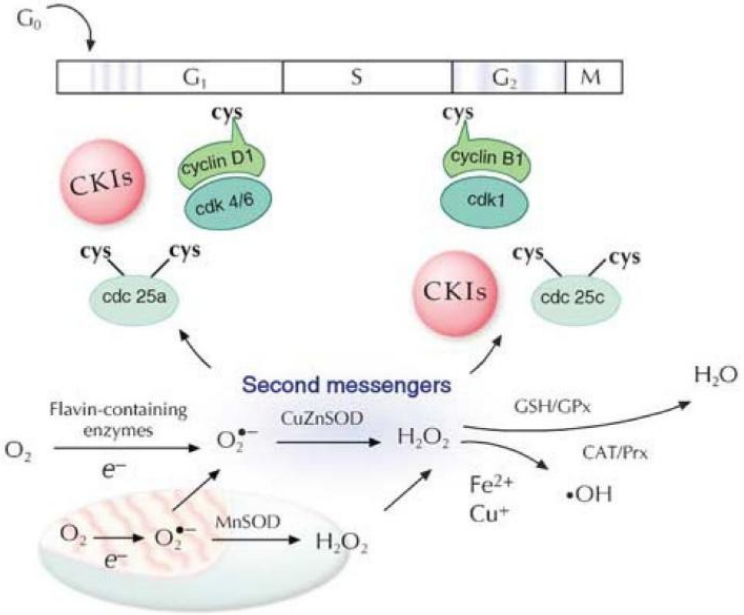
NRF2 TRANSCRIPTION FACTOR CONTROLS METABOLIC PATHWAYS

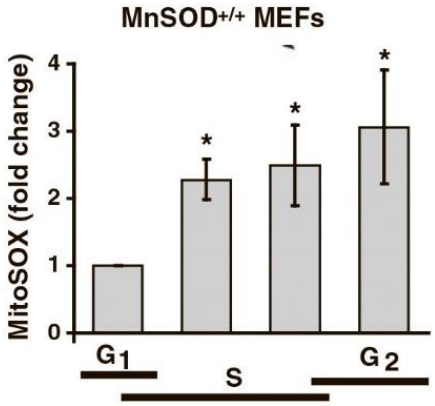


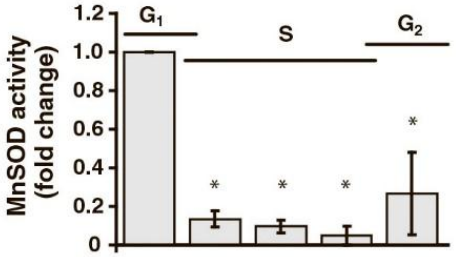
Hayes & Ashford, Cell Metab 2012

- Another transcription factors that are heavily implicated in control of metabolic pathways are HIF1a and c-Myc, both of which are activated by HCV
- A growing number of evidence suggest that metabolic changes in the cell can trigger tumour progression

ROS CAN AFFECT CELL CYCLE (EXPERIENCE FROM HEPATITIS-UNRELATED STUDIES)

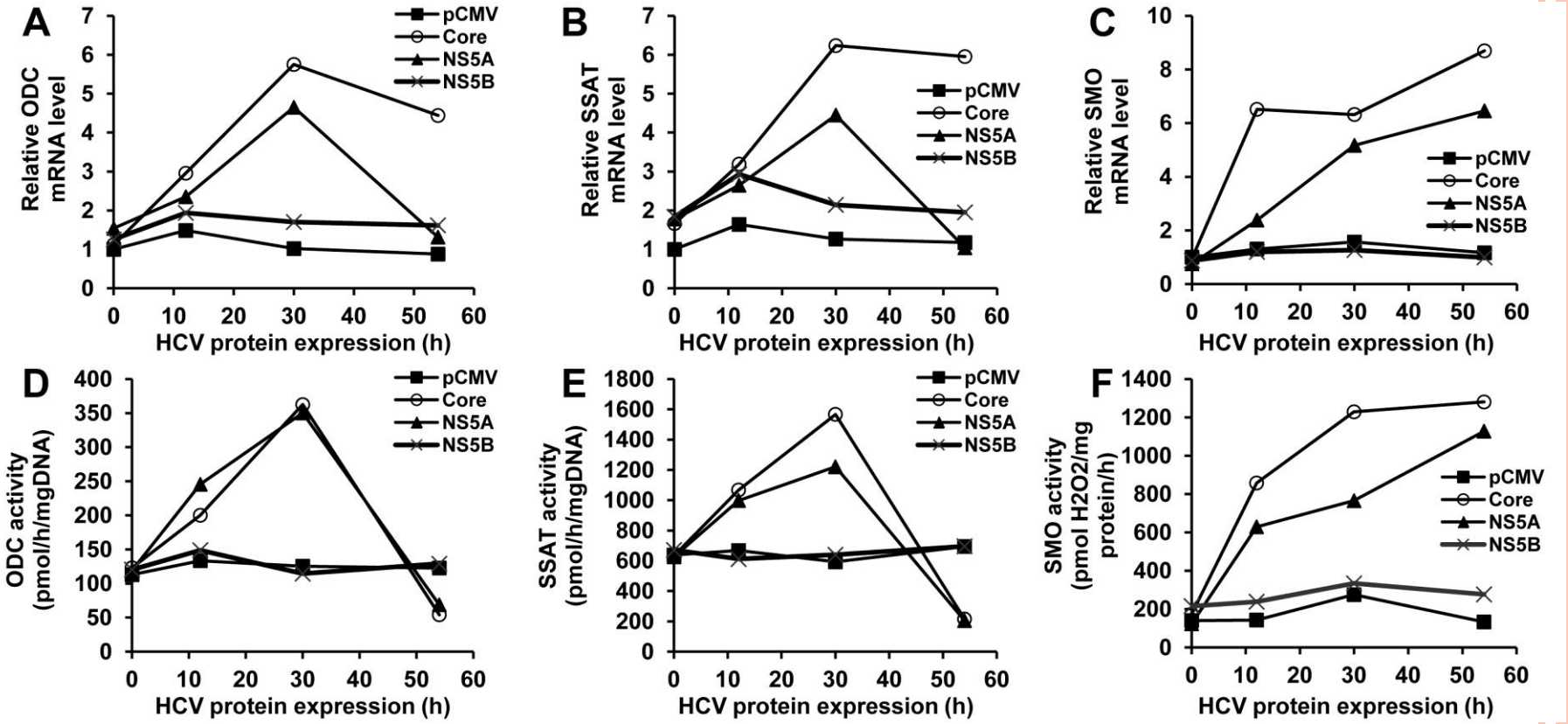






Adapted from *Menon & Goswani et al., Oncogene 2007, Sarsour et al, Cancer Res 2012*

INTERPLAY BETWEEN BIOGENIC POLYAMINES AND HCV-INDUCED OXIDATIVE STRESS



ODC SSAT SMO

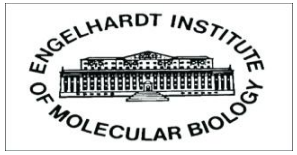
Summary

1. Hepatitis C (HCV) virus is a carcinogenic virus, elimination of infection does not reduce risk for development of liver cancer to the baseline level
2. HCV triggers massive oxidative stress as shown both in vitro and in vivo
3. Markers of oxidative stress correlate with liver inflammation, fibrosis score and risks for liver cancer
4. Reactive oxygen species (ROS) are contribute to tumorigenesis even in the absence of inflammation
5. ROS are involved in fibrogenesis
6. Induction of ROS in the infected cells contribute to dysregulation of signaling pathways, metabolic changes, genome instability etc

Future directions

1. Estimation of role of different ROS-producing systems in HCV-associated pathologies
2. Detailed analysis of compartmentalization of ROS production in the infected cells
3. Analysis of HCV impact of ROS-scavenging systems (such as peroxyredoxins)
4. Explore possible redox-sensitive modification of viral and host cell proteins and their impact in pathologies

Acknowledgements



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