Glutathione – Regulation

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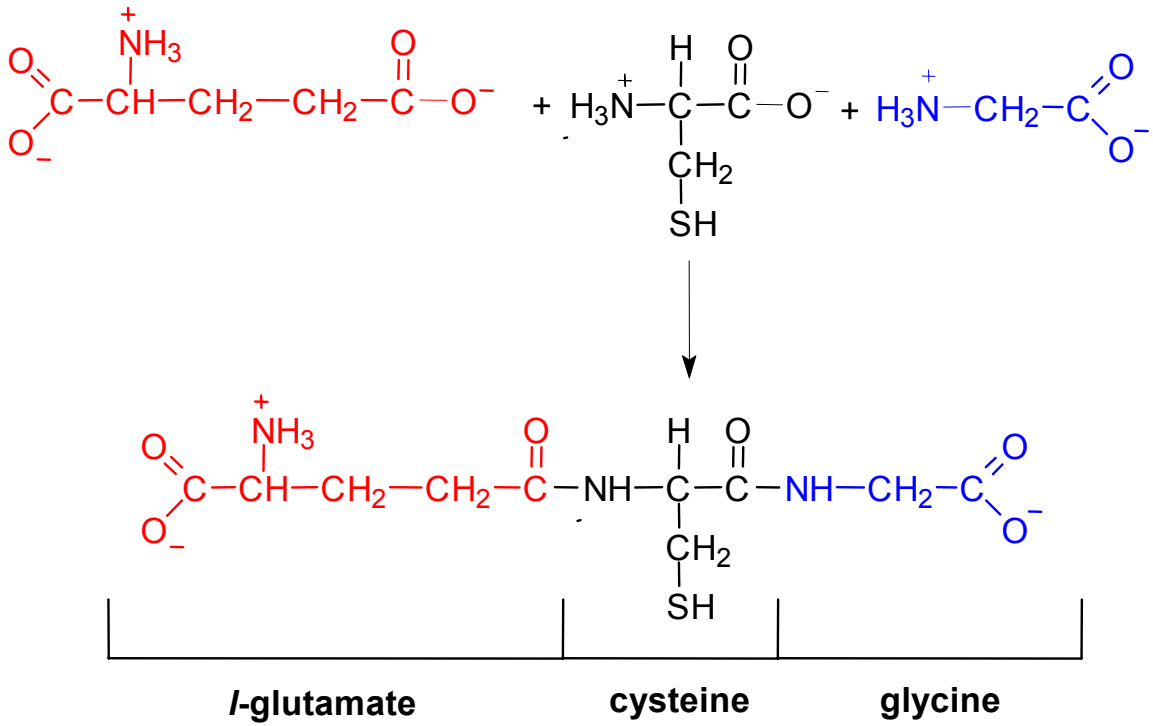
Nomenclature

- CFTR = cystic fibrosis transmembrane conductance regulator;
- GCL = glutamate cysteine ligase (sometimes called GCS);
- GCS = γ-glutamylcysteine synthetase (older name for GCS);
- GS = glutathione synthase (sometimes called glutathione synthetase);
- GSH = glutathione, also referred to as reduced glutathione;
- GSSG = glutathione disulfide, also referred to as oxidized glutathione;
- GST = Glutathione-S-transferase;
- MRP = multi-drug resistance protein;

Structure of GSH



Importance of glutathione and why it needs to be regulated

- GSH is the most abundant non-protein thiol in the cell;
- It serves antioxidant and cytoprotective functions in the cell;
- It is critical in maintaining the redox environment of the cell;
- Regulation of GSH is essential. Cellular GSH is increased in times of stress, and down-regulated after a challenge has been faced.

GSH content is responsive to endogenous and exogenous stimuli

- Heat shock
  - Kondo *et al.*, *J Biol Chem*, 268: 20366; 1993.
- Heavy metal exposure
  - Woods and Ellis, *Biochem Pharmacol* **50**: 1719; 1995.
- Dietary factors
  - Dickinson *et al.*, *FASEB J* **17**: 473; 2003.
- Shear stress
  - Hermann *et al.*, *Arterioscler Thromb Vasc Biol* **17**: 3588; 1997.
- High glucose
  - Urata *et al.*, *J Biol Chem* **271**: 15146; 1996.
- Lipids and lipid products
  - Moellering *et al.*, *Biochem J* **362**: 51; 2002.
  - Dickinson *et al.*, *Free Radic Biol Med* **33**: 974; 2003.
- Oxidative/nitrosative stress
  - Shi *et al.*, *Am J Physiol* **267**: L414; 1994.
- Hormones, rapid liver growth and cancer
  - Huang *et al.*, Hepatology **27**:147; 1998; *FASEB J* **15**: 19; 2001.
- HIV
  - Buhl *et al.*, *Lancet* **2**: 1294; 1989.

The intracellular content of GSH is a balance between depletion and synthesis

- **Depletion**
  - Conjugation to compounds by GSTs
  - Peroxidase reactions
  - Export *via* MRP in some cells, and by the CFTR protein in tracheobronchial cells
- **Replenishment**
  - Salvage pathway
  - Direct uptake
  - *de novo* synthesis

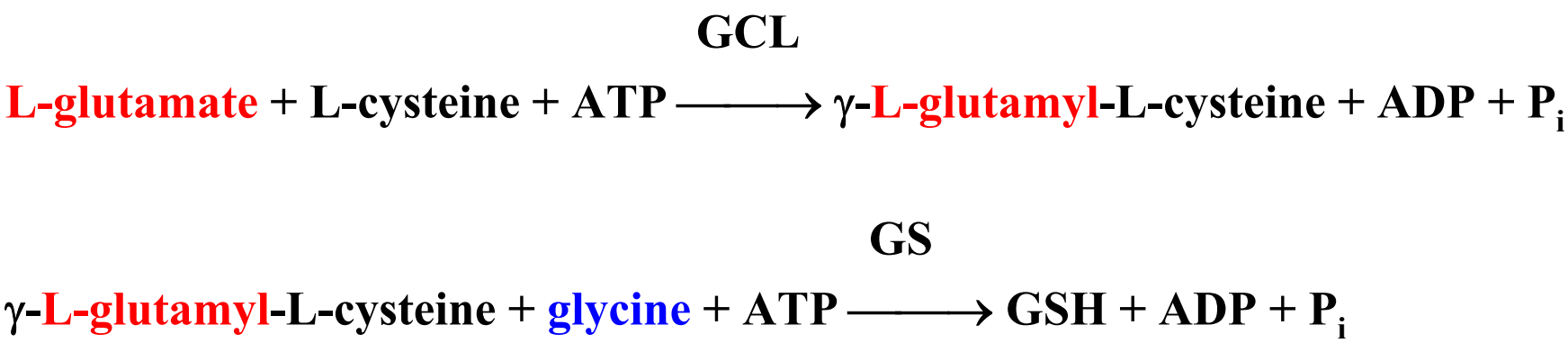
The rate of GSH synthesis is dependent on:

- **Cysteine availability**
- **Activity of pre-existing glutamate-cysteine ligase (GCL)**
- **Synthesis of new GCL**
  - Increased rate of translation
  - Increased stability of mRNA
  - Increased synthesis of mRNA

De novo synthesis of GSH

- Enzymatic synthesis occurs from the component amino acids (glutamate, cysteine, and glycine) *via* the sequential action of two ATP-dependent, cytosolic enzymes.
- The first enzyme is glutamate-cysteine ligase (GCL, E.C. 6.3.2.2) and combines glutamate and cysteine. It is generally rate-limiting.
- The second enzyme is glutathione synthase (GS, E.C. 6.3.2.3), which adds glycine yielding GSH.

De novo synthesis of GSH

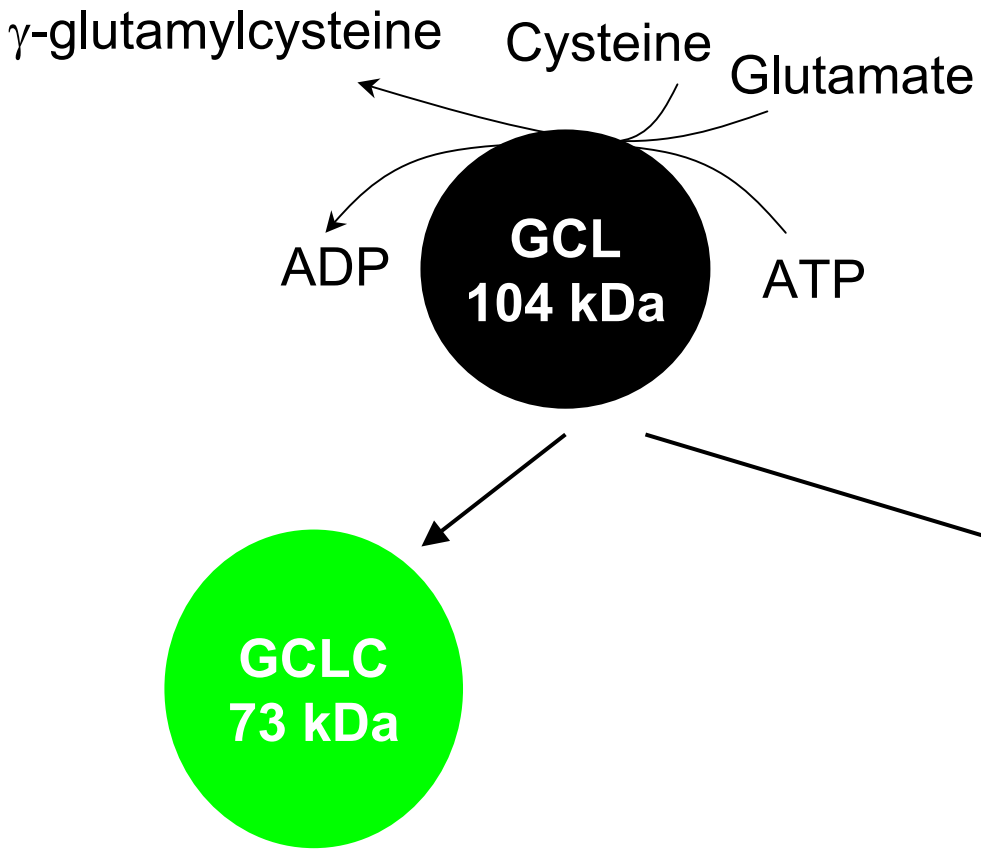


where:

GCL: glutamate cysteine ligase, sometimes called γ-GCS;

GS: glutathione synthase

Composition of the GCL holoenzyme



GCL is a heterodimer that can be separated under non-denaturing conditions to yield two subunits, GCLC and GCLM.

- Seeling *et al.*, *J Biol Chem* **259:** 9345; 1984.

- **catalytic activity**
- **site of GSH feedback inhibition**

- **regulates activity of holoenzyme**
  - **reducing the K_m for glutamate**
  - **elevating the K_i for GSH**

Roles of GCLC and GCLM

- **GCLC is the 'heavy' subunit (~73 kDa) and has the catalytic activity. It is the site of GSH feedback inhibition.**
  - Seeling et al., *J Biol Chem.* **259:** 9345; 1984.

- **GCLM is the 'light' (~28 kDa), or modulatory subunit. When in association with GCLC it regulates activity by reducing the K_m for glutamate and elevating the K_i for GSH, thereby making the enzyme more efficient and less sensitive to feedback inhibition.**
  - Tu and Anders, *Arch Biochem Biophys.* **354:** 247; 1998.
  - Choi *et al.*, *J Biol Chem.* **275:** 3696; 2000.

Regulation of the GCL holoenzyme

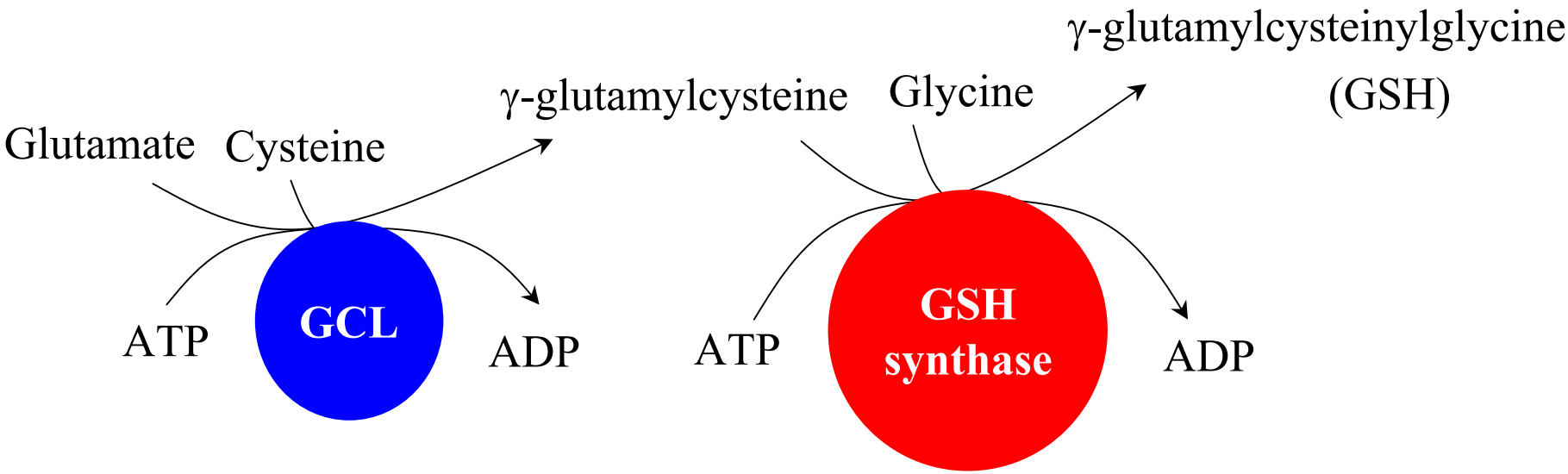
- The GCL holoenzyme can be regulated by S-nitrosation, phosphorylation, and oxidation.
  - Griffith, *Free Radic Biol Med*, **27:** 922; 1999.
  - Sun *et al.*, *Biochem J*, **320:** 321; 1996.
  - Ochi, *Arch Toxicol*, **70:** 96; 1995.

  Increased GCL activity is usually due to increased content of the GCL subunits, GCLC and GCLM, often a result of increased gene expression.

Glutathione Synthase

- GS is a homodimer of ~118 kDa.
- Much less is known about the role GS plays in regulating intracellular GSH. Recent studies suggest that GS and GCL are coordinately regulated and that GS induction can further enhance GSH biosynthesis.
  - Huang *et al.*, *BBA* **1493**: 48; 2000; *FASEB J* **15:** 19; 2001.
  - Njalsson *et al.*, *Biochem J* **349:** 275; 2000.
  - Yang *et al.*, *JBC* **277**: 35232, 2002.

Formation of GSH from GCL and GS



GCL combines glutamate and cysteine, at the expense of ATP, to form γ-glutamylcysteine, which is then combined with glycine by GS, again at the expense of ATP, to yield GSH.

Induction of *Gcl* gene expression

- **Increased activity of GCL leading to increased GSH content is generally a result of increased mRNA content of the *Gcl* genes, *Gclc* and *Gclm*.**
- **Both increased mRNA stability and transcription can be involved, as has been clearly demonstrated for the bioactive lipid 4-hydroxynonenal (4HNE)**
  - Liu *et al.*, *Am J Physiol* **275:** L861; 1998.

  although the relative roles of these two distinct mechanisms is often not clearly reported.

Enhancer elements and *Gcl* expression

- The promoter regions for *Gclc* and *Gclm* have been cloned, sequenced, and analyzed for putative enhancer elements:
- *Gclc*
  - Human Mulcahy *et al.*, *J Biol Chem* **272:** 7445; 1997.
  - Rat Yang *et al.*, *Biochem J* **357:** 447; 2001.
  - Mouse Bea *et al.*, *Circ Res* **92:** 386; 2003.

    (demonstrates functional EpRE elements; not a complete sequence)
- *Gclm*
  - Human Moinova and Mulcahy, *J Biol Chem* **273:** 14683; 1998.
  - Rat Yang *et al.*, *Biochem J* **391:** 399; 2005.
  - Mouse Hudson and Kavanagh, *Biochim Biophys Acta* **1492:** 447; 2000.

Enhancer elements for *Gclc* and *Gclm*

- Many publications have reported the necessity of specific enhancer elements in the regulation of the *Gcl* genes in response to specified conditions. Typically these have been demonstrated using reporter construct analysis and with gel shift assays (electrophoretic mobility shift assays, EMSAs).
- Those elements having garnered the most attention and support are TRE (AP-1 binding) and EpRE (electrophile response element, also know as the antioxidant reponse element, or ARE). Although, a couple of reports suggest a role for NFκB in mediating *Gclc* expression (the κB element is absent in the human *Gclm* promoter).

Correlation between hepatic *Gcl* expression and *cis*-acting element binding

Condition	Expression of *Gclc*	Expression of *Gclm*	Binding to EpRE	Binding to AP-1	Binding to NFκB
Human HCC	↑	NC	↑	↑	↑
Rats: Ethanol	↑	NC	↑	↑	↑
Thioacetamide	↑↑	↑↑	↑	↑	↑

HCC = hepatocellular carcinoma
NC = no change

Hepatology **30**: 209; 1999.
Toxicol Appl Pharmacol **159**: 161; 1999.
FASEB J **15**: 19; 2001.

Role of EpRE and AP-1 in *Gclm* transcriptional regulation

- Increased nuclear binding to EpRE and AP-1 occurs in human HCC despite lack of increased *Gclm* expression.
- There is also a lack of correlation between inducible *Gclm* expression and nuclear binding activity to EpRE or AP-1 in the rat.
- The role of EpRE and AP-1 in inducible *Gclm* expression *in vivo* remains unclear.

Binding activity and transcriptional activity

- Increased DNA binding activity, as determined by EMSA analysis, demonstrates increased binding of transcription factor complexes to a specific enhancer element. But it has been reported that increased binding does not necessarily increase the rate of transcription.

- AP-1 and EpRE binding complexes are dimers, and can contain many different transcription factor proteins.

- Some of these enhance transcription, while others suppress transcription. So in addition to the overall DNA binding activity, the composition of the complex is important in determining gene expression.

Curcumin alters *Gcl* expression in HBE1 cells through AP-1 and EpRE elements

- Curcumin has many adaptive properties, and routine dietary intake is correlated with decreased risks for colon and prostate cancers. It has been used in folk medicine for over 1000 years for its healing properties.

- Curcumin increases intracellular GSH through *Gcl* induction, mediated by increased binding to EpRE and AP-1 elements in both *Gclc* and *Gclm.*

- More importantly, immunodepletion studies (supershifts) determined that the composition of the complex changed in response to curcumin.
  - Dickinson *et al.*, *FASEB J* **17:** 473; 2003.

AP-1 and EpRE complex remodeling

- Upon curcumin exposure in HBE1 cells, AP-1 complexes were changed to include phosphorylated c-Jun, which is known to drive expression; basal AP-1 complexes had no detectable c-Jun.
- EpRE complexes had more JunD, phosphorylated c-Jun and Nrf 2, all positive regulators of transcription, and less MafG and MafK, negative regulators.
  - Dickinson *et al.*, *FASEB J* **17:** 473; 2003.
- Together these changes are consistent with increased activity of the binding complexes, and provide evidence that increased DNA binding activity alone may not always be sufficient, while modest increases in binding activity, when combined with more active binding complexes, could significantly increase expression.

Induction of *GS* gene expression

- Agents that induce both GCL subunits also induce *GS*.
- Agents or treatments that increase only *Gclc* do not increase *GS* with the exception of rapid liver growth, such as after partial hepatectomy or hepatocellular carcinoma.
  - Huang *et al.*, *BBA* **1493**: 48; 2000; *FASEB J* **15:** 19; 2001.

Regulatory *cis*-acting elements for *GS*

- AP-1 serves as an important enhancer for the rat *GS*. Treatment of H4IIE cells (a rat hepatoma cell line) with *tert*-butylhydroquinone strongly induces the expression of *GS* and the rat *GS* promoter by a mechanism that requires AP-1.

- NF-1 serves as a repressor for the rat *GS*. Mutating the two putative NF-1 binding sites of the rat *GS* promoter resulted in a significant increase in promoter activity.
  - Yang *et al.*, *J Biol Chem* **277:** 35232; 2002.

Summary

- Regulation of GSH synthetic capacity can occur at many levels including regulation of *Gclc*, *Gclm*, or *GS*, either in a coordinated manner or individually.
- Regulation can occur transcriptionally as well as post-transcriptionally.
- A better understanding of how these enzymes are regulated will improve our ability to modulate the GSH synthetic capacity for therapeutic purposes.