ICSB2025 (December 6-7, 2025)

- The 14th International Workshop on Cancer Systems Biology
- https://sysbio.med.sustech.edu.cn/

12月6日（上午）

Session	时间	内容	主讲人
开幕式	8:30–8:50	开幕式	孙之荣（清华大学） 徐鹰（南方科技大学）
	8:50–9:00	合影	
Session1	9:00–9:50	主题报告：Metabolic Regulation of Cancer and Immunity	吕志民（浙江大学）
	9:50–10:40	主题报告：Genetic Dissection of Chromatin Regulatory Network	Chao Lu（哥伦比亚大学）（线上）
Session2	10:50–11:40	主题报告：Mesenchymal Stem Cells and Inflammatory Tumor Environment	时玉舫（苏州大学）（线上）
	11:40–12:30	主题报告：Quantifying the immune temperature of cancer cells with spatial omics	Jiguang Wang（香港科技大学）

12月6日（下午）

Session	时间	内容	主讲人
Session1	14:00–14:50	主题报告：Dynamical Systems Biology with AI	陈洛南（上海交通大学）
	14:50–15:40	主题报告：铁死亡、铁科学与重大疾病诊治	王福俤（浙江大学）
	15:40–16:30	主题报告：从化学稳态失衡/再平衡的角度解析阿兹海默症（关联脑血管疾病）的发生、发展	徐鹰（南方科技大学）
Session2	16:45–17:35	主题报告：3-D chromatin structure, methylation and gene expression correlation in cancer cells	高毅勤（北京大学）
	17:35–18:25	主题报告：To Be or Not to Be —— The Existentialism of PIK3CA Mutation in Lung Cancer	季红斌（西湖大学）

12月7日（上午）

Session	时间	内容	主讲人
Session1	8:00–8:50	主题报告：Gut Microbiome/Metabolites in MASLD and HCC via Gut–Liver Axis	Jun Yu（香港中文大学）
	8:50–9:40	主题报告：Exploring the pathogenesis and translational applications of common mental disorders based on multi-omics data	岳伟华（北京大学）
	9:40–10:30	主题报告：AI × Cancer Biology：Harnessing Big Data to Decode 3D Genome Architecture and Therapeutic Vulnerabilities	Melissa J. Fullwood（南洋理工大学）
Session2	10:40–11:30	主题报告：Systems biology approaches to deciphering prostate cancer regulation	Jindan Yu（埃默里大学）
	11:30–12:20	主题报告：Advancing computational tools and resources to study metabolic reprogramming in cancer	Chi Zhang（俄勒冈健康与科学大学）

12月7日（下午）

Session	时间	内容	主讲人
Session1	13:50–14:40	主题报告：Mesoscale intravital fluorescence microscopy	吴嘉敏（清华大学）
	14:40–15:30	主题报告：Adeno-to-squamous transition drives resistance to KRAS inhibition in LKB1 mutant lung cancer	Micheal Zhang（德克萨斯大学达拉斯分校）（线上）
	15:30–16:20	主题报告：Revolutionizing Target Discovery for Complex Diseases: A Deep Learning-Driven Approach	谢正伟（北京大学）
Session2	16:35–17:25	主题报告：Cross-kingdom regulation of host metabolic homeostasis by gut microbial enzymes and metabolites	姜长涛（北京大学）（线上）
	17:25–18:15	主题报告：Percolation and its function in biological systems	Zeyu Shen（香港科技大学）

Expectation of the Students

- Read the short book "Introduction to cancer biology" and the suggested literature
- Active participation in in-class discussions and no private in-class conversations
- Have your phones switched off and absolutely no phone calls in class!

肿瘤信息学

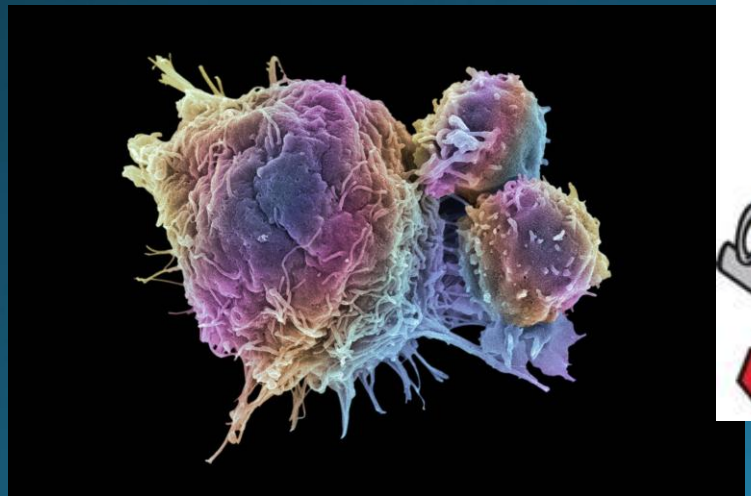
Cancer Biology: an informatics perspective

徐鹰

南方科技大学医学院

Lecture II

Disease vs. normal biology, evolutionary principle, cancer evolution, necessary environment, driving stressors, metabolic reprogramming



Two Main Schools of Thoughts

- Cancer is the result of genetic mutations

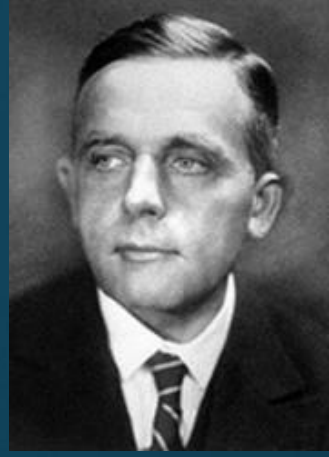
Bishop and Varmus, 1989
Nobel prize winners

- Cancer is the result of altered energy metabolism

Otto Warburg 1931
Nobel prize winner

Cancer is a Genomic Disease

- "Cancer as a genomic disease" has profoundly affected the field of cancer research
- When studying a cancer, a key priority is to identify oncogenes and tumor suppressor genes
- Many such genes have been suggested for a variety of cancers
  - APC mutation considered as the tumor suppressor gene of colorectal cancer
  - BRCA mutations as the tumor suppressor genes of breast cancer
  - Philadelphia chromosome as the oncogene of CML
  - .....



glucose

glucose-6-phosphate

glycolysis

oxidative phosphorylation

lactate

pyruvate

produces 2 ATPs per glucose

acetyl CoA

mitochondria

produces 36 energy units (ATPs) per glucose

TCA cycle

electron transport chain

Cancer is a Metabolic Disease

- Warburg effect: cancer cells produce energy by glycolysis followed by lactate fermentation in cytosol, rather than by oxidation of pyruvate in mitochondria in normal cells even when cells have oxygen

- Otto Warburg postulated in mid 1930's that this change in energy metabolisms is the fundamental cause of cancer, and hence considered cancer as a disease of metabolism

- In 1960's, Warburg stated: Cancer … has countless secondary causes; but there is only one prime cause, (which) is the replacement of respiration of oxygen in normal body cells by a fermentation of sugar.

- There are so many unanswered questions about cancer biology and medicine, including some very basic ones
  - People with the same cancer type but different outcome
  - Why men have higher cancer rates than women in general
  - Why cancers in certain organs are more malignant than those in other organ types
  - Why pediatric cancers are generally easier to treat than adult ones
  - Why metastasized cancers are more malignant than the primary counterparts
  - .....
- Our understanding about and ability to treat cancer is so embarrassingly limited

Where Do We Go From Here?

- We need to have a general framework through which all the pieces of knowledge about cancer can be integrated and all the omic data can be adequately explained
- The framework should allow us to study cancer as an evolutionary problem, driven by various types of identifiable pressures
- It should enable us to examine cancer in a more holistic manner rather than the traditional reductionist views and approaches

Prelude

- The chemical-physical microenvironments, the platform for executing cellular chemistry, are fundamentally different between healthy and disease biology

- Hence, we need to have new ways to look at the disease data and build disease models differently than how we have been studying biology under physiological conditions!

生物体系在演化过程中的保守性

- 相关的物种之间有很强的保守性，包括同源基因、生物过程及网络、以及细胞基础设施，即便其演化距离很远。
- 利用这一性质，通过对简单生物（称为模式生物）的研究，学者们可以很快获得大量有关人类生物学的信息：
  - 人的代谢系统中很大一部分来自于对酵母代谢系统的知识
  - 人的神经系统中的一大部分来自于对线虫神经系统的了解
  - 人的发育系统中的一大部分来自于对果蝇发育系统的研究
  - 。。。

疾病生物学不同于正常生物学

- 在过去50年，人们发现可以在很大程度脱离开基本的化学物理环境来研究细胞生物学，而仅需考虑生物大分子（蛋白、RNA）之间的相互作用，且取得了巨大的成功
- 一个重要的底层原因是：模式生物细胞与相关的人类细胞有着高度相似的化学物理微环境，造成一个“错觉”：生物学就是生物分子的网络
- 而这一相似的微环境在疾病细胞与健康细胞之间基本不存在：两者之间存在巨大的差别
- 这使得通过同源保守性获得大量信息的方式很难用于疾病的研究

健康与肿瘤细胞理化环境的差异

正常细胞

- 细胞浆 pH: 6.8 – 7.2.
- 细胞间液 pH: 7.3 – 7.5
- 细胞内及外主要电解质浓度:
  - 钠离子: 10 : 140
  - 钾离子: 145 : 4
  - 细胞外膜电势: 70 mV
- 线粒体膜电势: 140 mV

肿瘤细胞

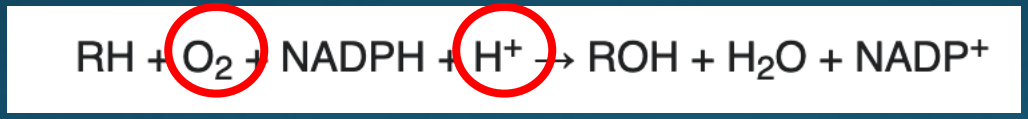
- 细胞浆 pH: 7.2 – 7.5 .
- 细胞间液 pH: 6.4 – 6.6
- 细胞内及外主要电解质浓度:
  - 钠离子: 60 : 140
  - 钾离子: 145 : 5
- 细胞外膜电势: 27 mV
- 线粒体膜电势: 210 - 280 mV

- 肿瘤微环境缺氧，且越缺氧、越恶性的肿瘤
- 肿瘤微环境有高氧化态:氧化能力> 还原能力
- 。。。

底层化学物理条件改变的影响

- **例1**:当细胞内缺氧时，耗O_2的化学反应就会被抑制。同样地，当细胞浆受到碱性压力（pH过高）时，消耗氢离子的反应就会被抑制。

- 因而当两者都低到一定程度时，肿瘤细胞就会耐药，因为药物代谢的一组酶是P450家族的酶，其反应的一般形式为：



- 从药物敏感到耐药的转化，并不需要特别调控或突变，因为当底物浓度下降时，酶的表达会跟着下降 （底物的浓度决定酶的表达量）

底层化学物理条件改变的影响

- 例2：肿瘤细胞浆的pH比正常细胞的要高。一个流行的观点是肿瘤细胞使用Na^+/H^+交换器NHE1持续将H^+泵出细胞，导致胞内碱性化。
- 对正常细胞，从反应热力学的角度，这有可能

$$\Delta G_{Na^+} + \Delta G_{H^+} = 8.31 \times 310 \times \left(\ln\left(\frac{12}{140}\right) + \ln\left(\frac{10^{-6.6}}{10^{-7.4}}\right)\right) = 8.31 \times 310 \times (-0.615) = -1583J$$

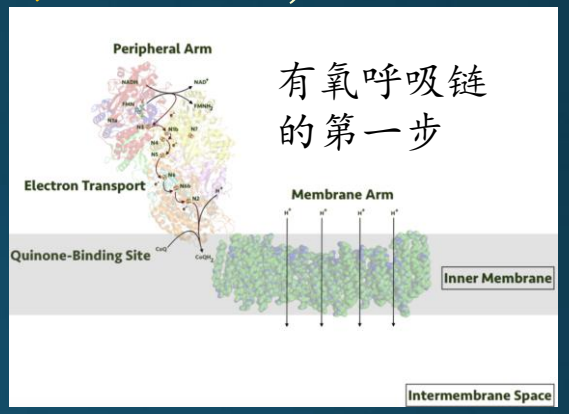
- 但在肿瘤中，这不大可能，因为肿瘤细胞内Na^+的浓度远高于正常细胞，从10:140 mmol/L 增加到 60:140 mmol/L

$$\Delta G_{Na^+} + \Delta G_{H^+} \geq 8.31 \times 310 \times \left(\ln\left(\frac{59}{140}\right) + \ln\left(\frac{10^{-6.6}}{10^{-7.4}}\right)\right) = 8.31 \times 310 \times 0.978 = 2519J$$

底层化学物理条件改变的影响

- **例3**: 肿瘤的线粒体内膜电势比正常细胞的高50-100%，

$$NADH + H^+ + CoQ + 4H^+_{in} \rightarrow NAD^+ + CoQH_2 + 4H^+_{out}$$



- 这导致电子传递链将氢离子，从线粒体基质推到线粒体内膜之外的难度增加、因此效率下降到正常的 < 10%

- 进而导致线粒体内膜两侧的氢离子浓度差下降，使得产生ATP的效率下降

- 我们需要新的思维方式及分析框架、技术来研究肿瘤及其它疾病生物学

It is hard to be a cancer but why?

- Cancer tissue cells consume up-to 20 to 30-fold more glucose than normal matching cells
- They require significantly more ATPs than normal cells
- Their levels of stresses, e.g., ER stress, are significantly higher than the normal cells
- Over 97% of cancer cells die soon after they are created

- Hence, energetically it does not seem to make sense for normal human cells to take the route to become cancerous.

- Question: why do they still do it?

A Major Hypothesis

- all such cells are under the same yet-to-identify stressors that they must take the cancerous route since otherwise they will die 100%?

生物演化的法则

- 关于微生物的演化，学者们早已建立了以下广为微生物学者接受的演化框架（在压力下，适者生存）：

生存压力 → 基因突变/表观改变 → 有些被选择来启动代谢重编程以适应压力

- 这些压力可能包括物种之间的竞争，微环境的改变等
- 同样地，这一框架也被广泛用于研究植物的演化，如植物如何适应土壤的干旱、水涝、虫害等

- 但人类疾病学者似乎不愿意接受这一观点，认为“基因突变”是肿瘤发生的终极原因

Red Queen Hypothesis: an example

- Paterson *et al.* designed two experiments to study coevolution of the bacterium *Pseudomonas* and its antagonistic phage Φ2
  - Have them evolve to a dynamic equilibrium
  - Then give one a slight competitive edge and keep the other unchanged
- The finding is that the equilibrium shifts towards increased population of the organism with an advantage, leading to the evolution of the other one till the original equilibrium is regained
- They concluded that antagonistic coevolution is a cause of rapid and divergent evolution, and is likely to be a major driver of evolutionary change

Paterson et al. Nature 2010

如果我们应用这一框架来研究肿瘤

- 什么是驱动肿瘤发生、发展的生存压力？
- 压力可能较难直接研究，那就研究肿瘤中保守的代谢重编程

- 肿瘤喜欢使用从头合成的方式产生核苷酸，尽管细胞可从血液中回收核苷（加磷酸化）。我们发现：越恶性的肿瘤，从头合成核苷酸的比例越高
- 肿瘤中的氨基酸代谢高度活跃，但释放氨基酸代谢废物氨气的尿素循环在所有（TCGA）肿瘤中都被抑制
- 肿瘤使用低效的发酵方式，而不是正常的、更高效的呼吸链产生ATP（瓦博格效应）

肿瘤研究的信息来源

- 我们系统地分析了**TCGA数据库**中的33种肿瘤，～2万个肿瘤组织的转录数据 – 这是世界上最大的肿瘤组织数据库
- 从每个肿瘤组织，获取～65000个基因的每个基因的表达数据，其反映了一个基因的活跃程度
- 一个基因在肿瘤中的表达值比对应的正常组织中的值高，就说这个基因上调了；如果低，就下调了

- 我们特别关心与pH有关的每个酶促化学反应

消耗一个氢离子

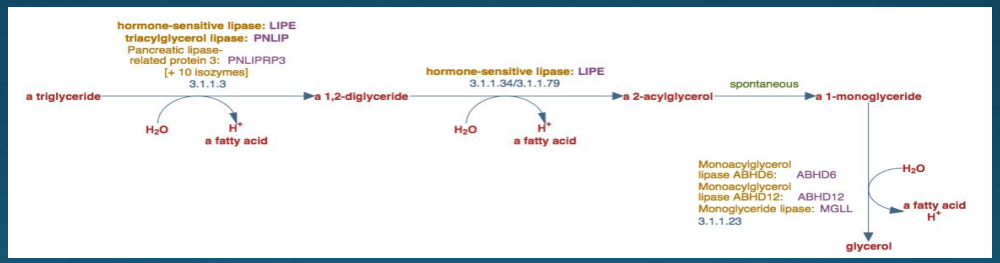
$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

P450酶催化的反应

HumanCyc 数据库

代谢重编程：核心技术

- 代谢通路：细胞将食物转化为能量、生物分子（DNA、RNA、蛋白、多糖、脂肪、有机酸等）（或反之）、及排出废物的化学反应过程，多由酶促反应来实现
- 这些通路都经过长期演化的选择、优化。一个通路的全部基因可通过某种调控方式被共同启动来完成一个将化合物A 转化成B的过程

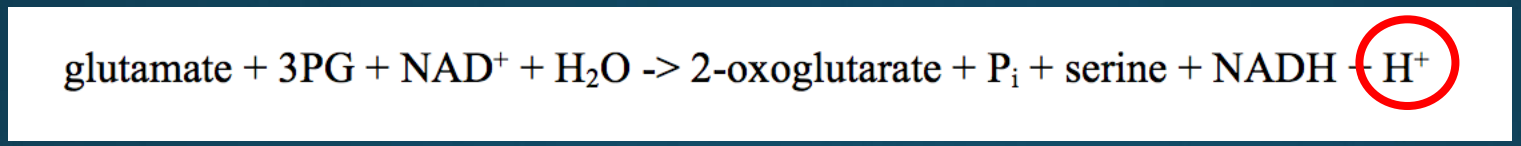


- 代谢重编程：在持续压力下，细胞能通过某些机制组装新的代谢通路，选择其中的某些来帮助细胞适应压力

- 肿瘤中的代谢重编程

丝氨酸从头合成

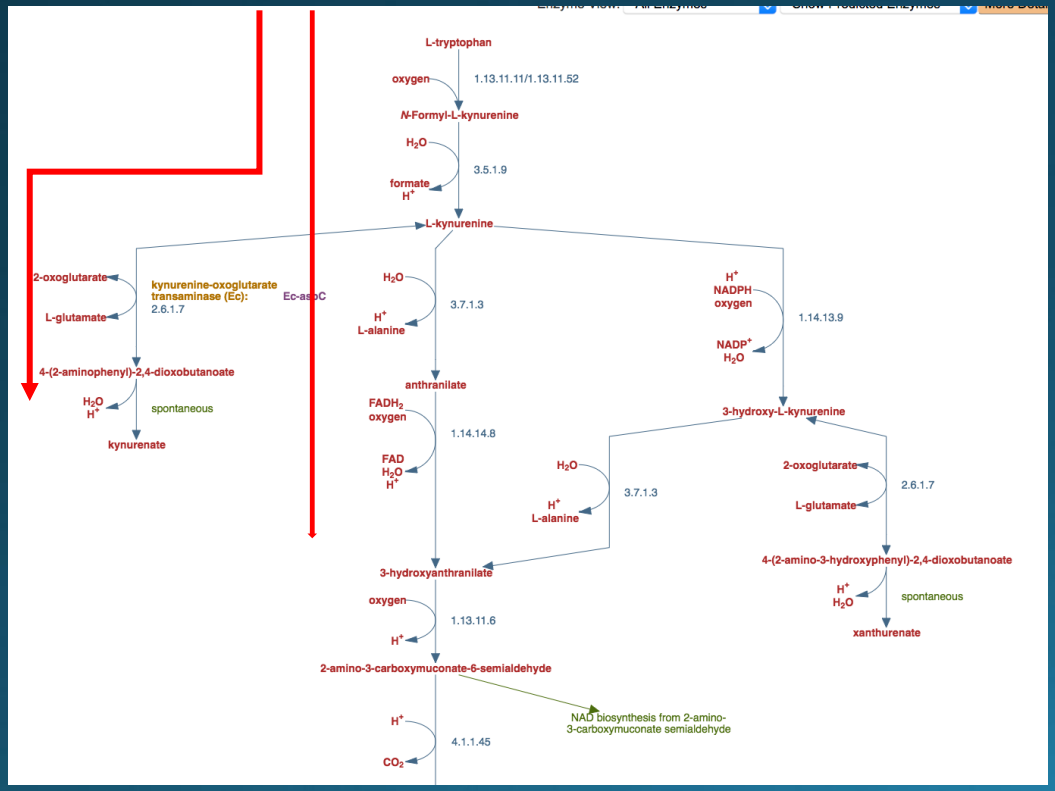
- 肿瘤生长需要丝氨酸，本可从血液中摄取，但肿瘤更倾向于自己从头合成；而且肿瘤的恶性程度越高，自己合成的比例越高



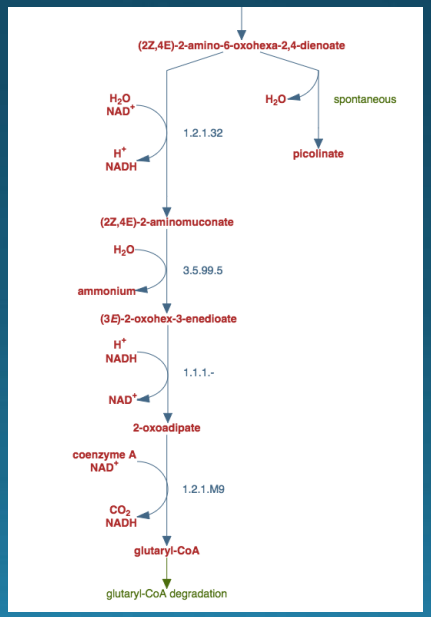
- 从头合成比从血液中摄取多产生一个H^+

Tryptophan Degradation in Cancer

- Normal cells degrade tryptophan to acetyl-CoA via the following pathway but cancer only uses part of the pathway to produce kynurenine and 3-hydroxyanthrranliate



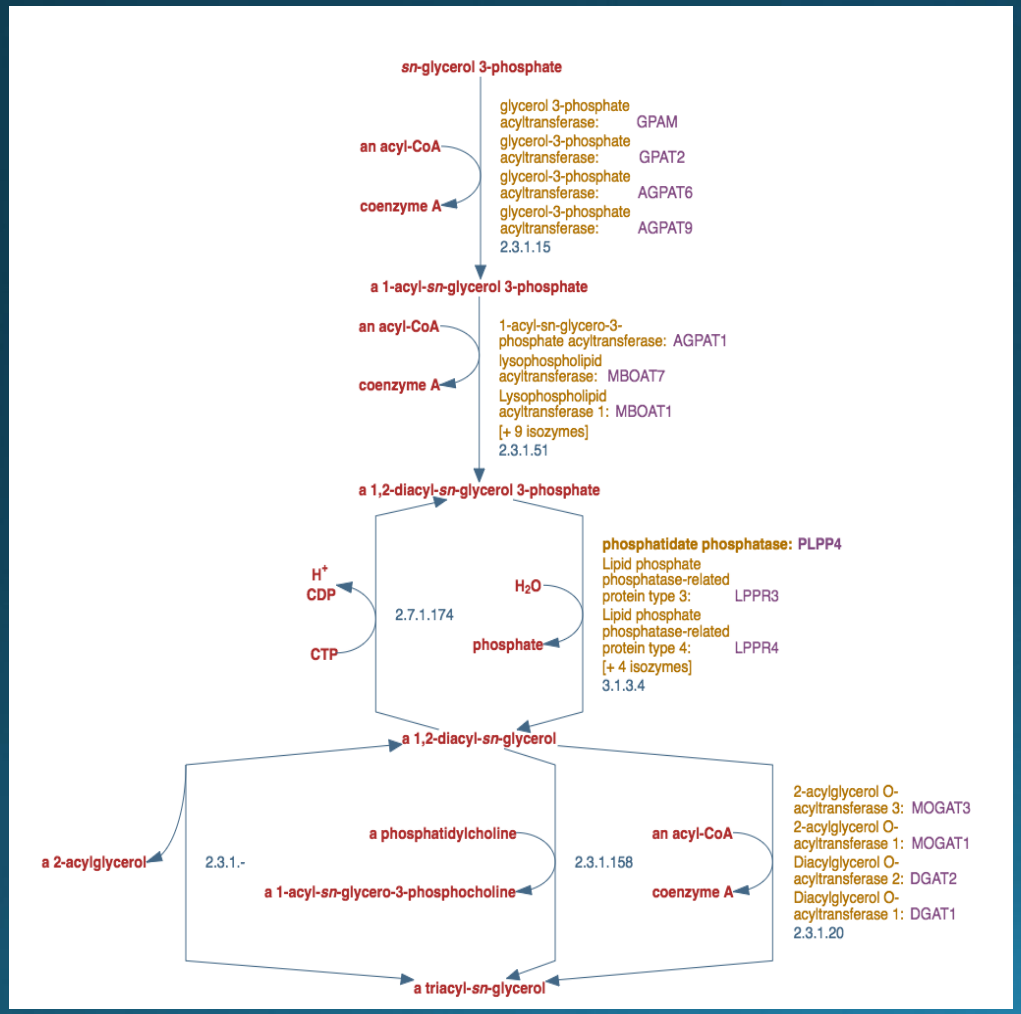
It produces the most number of protons

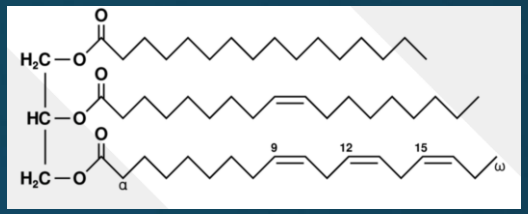


acidification

Increased Triglyceride Synthesis

- Cancer increases the activity of triglyceride synthesis





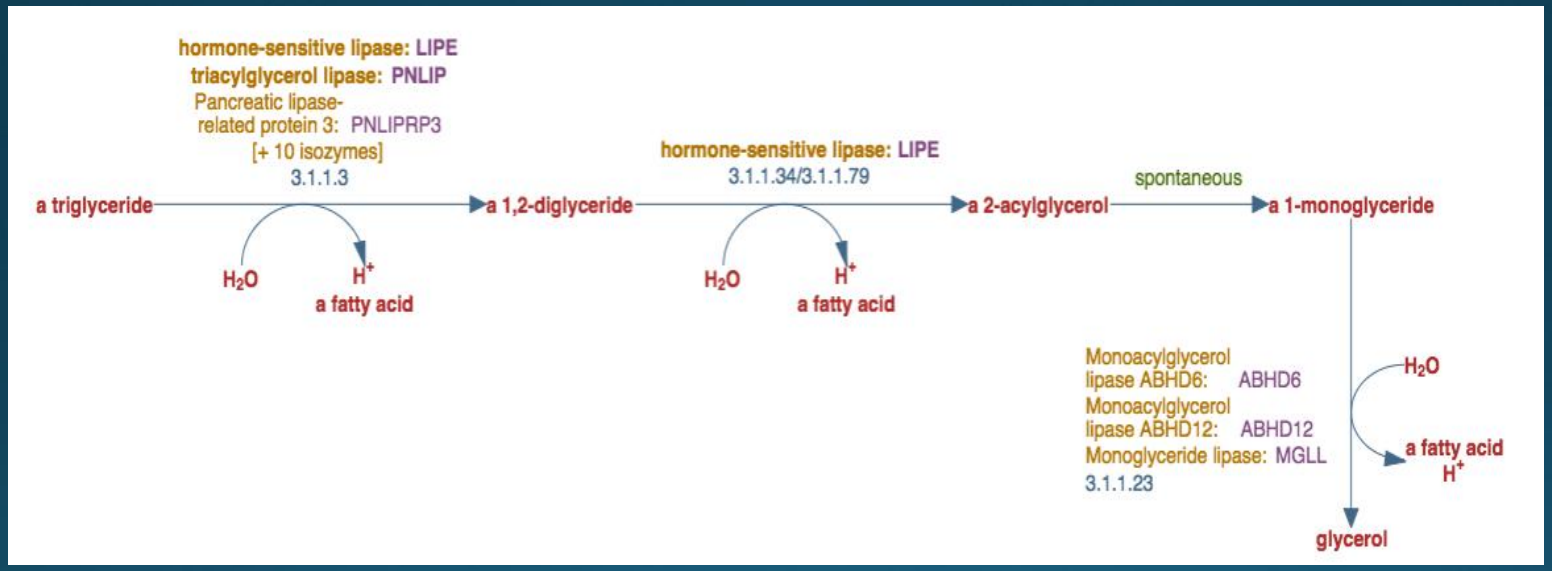
The process produces protons as long as CTP is available

Very interestingly, cancer has long been known to over-produce CTPs

acidification

Increased Triglyceride Degradation

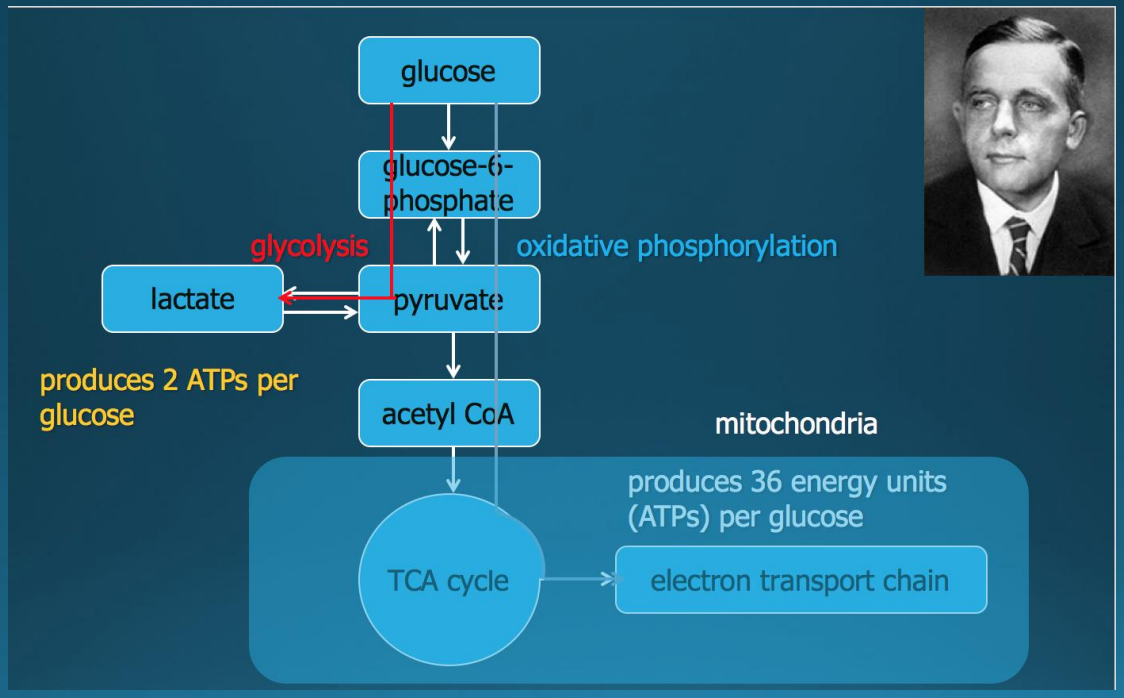
- Cancer also increases the activity of triglyceride degradation



acidification

Warburg Effect

- This ATP is different from that ATP: each ATP produced by Warburg effect produces one H^+ when the ATP is hydrolyzed while ATP produced by respiration is pH neutral!



- ATP generation by respiration
- $ADP^{3-} + HPO_4^{2-} \longrightarrow ATP^{4-} + OH^-$

- ATP generation by glycolysis
- $glucose + 2ADP^{3-} + 2HPO_4^{2-} \longrightarrow 2\ lactate + 2\ ATP^{4-}$

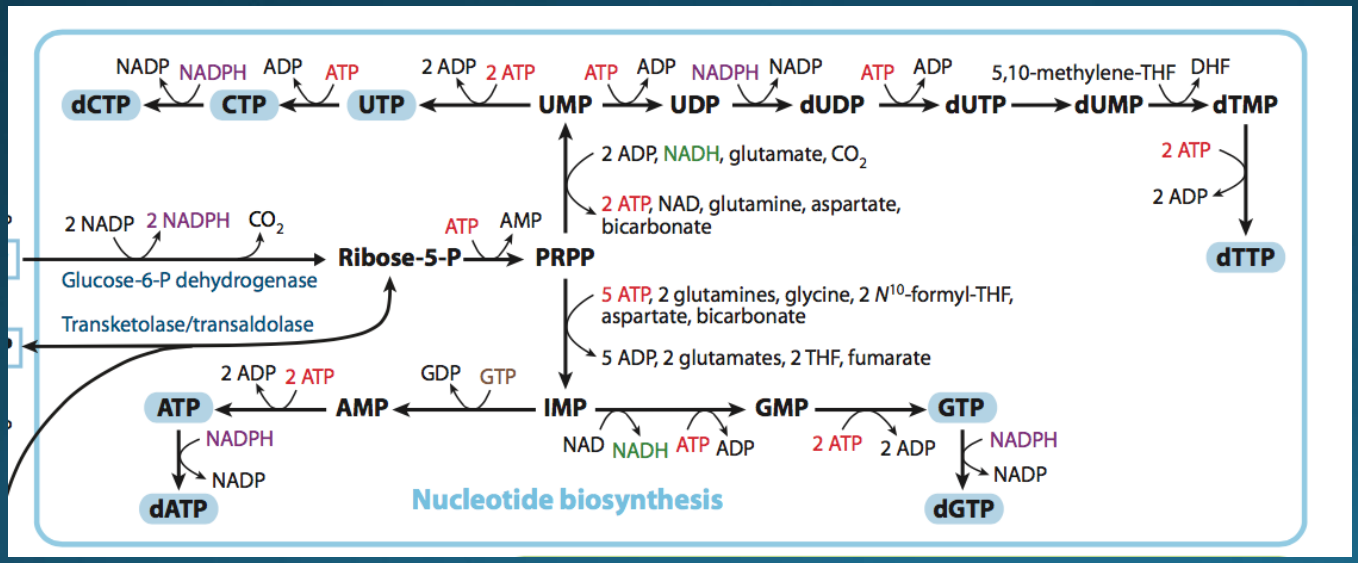
- Hydrolysis of ATP
- $ATP^{4-} + H_2O \longrightarrow ADP^{3-} + HPO_4^{2-} + H^+$

Warburg effect produces more protons than respiration

Nucleotide Metabolic Reprogramming

- Cancer tends to *de novo* synthesize nucleotides rather than uptake via salvage

- Cancer generally synthesizes considerably more purine and pyrimidine



purine

pyrimidine

Pyrimidine *de novo* synthesis:

5 ATP + 2 H_2O + 2 glutamine + aspartate + 5-phospho-D-ribose-diphosphate -> dCDP + H_2CO_3 + 5 H^+;

5 ATP + H_2O + glutamine + aspartate + 5-phospho-D-ribose-diphosphate -> dTDP + H_2CO_3 + 3 H^+.

Pyrimidine salvage pathway:

2 ATP + H_2O + 2-deoxycytidine -> dTDP;

2 ATP + 2-deoxycytidine -> dCDP + H^+;

2 ATP + thymidine -> dTDP + H^+.

Purine *de novo* synthesis:

5 ATP + GTP + H_2CO_3 + glycine + 2 aspartate + 5-phospho-D-ribose-diphosphate -> dADP + 8 H^+;

5 ATP + NAD^+ + H_2CO_3 + H_2O + glycine + aspartate + glutamine + 5-phospho-D-ribose-diphosphate -> dGDP + NADH + 9 H^+.

Purine salvage pathway I:

ATP + 2-deoxyadenosine -> dAMP + H^+;

ATP + 2-deoxyguanosine -> dGMP + H^+;

Purine salvage pathway II:

2 ATP + adenosine -> dADP + H_2O + H^+;

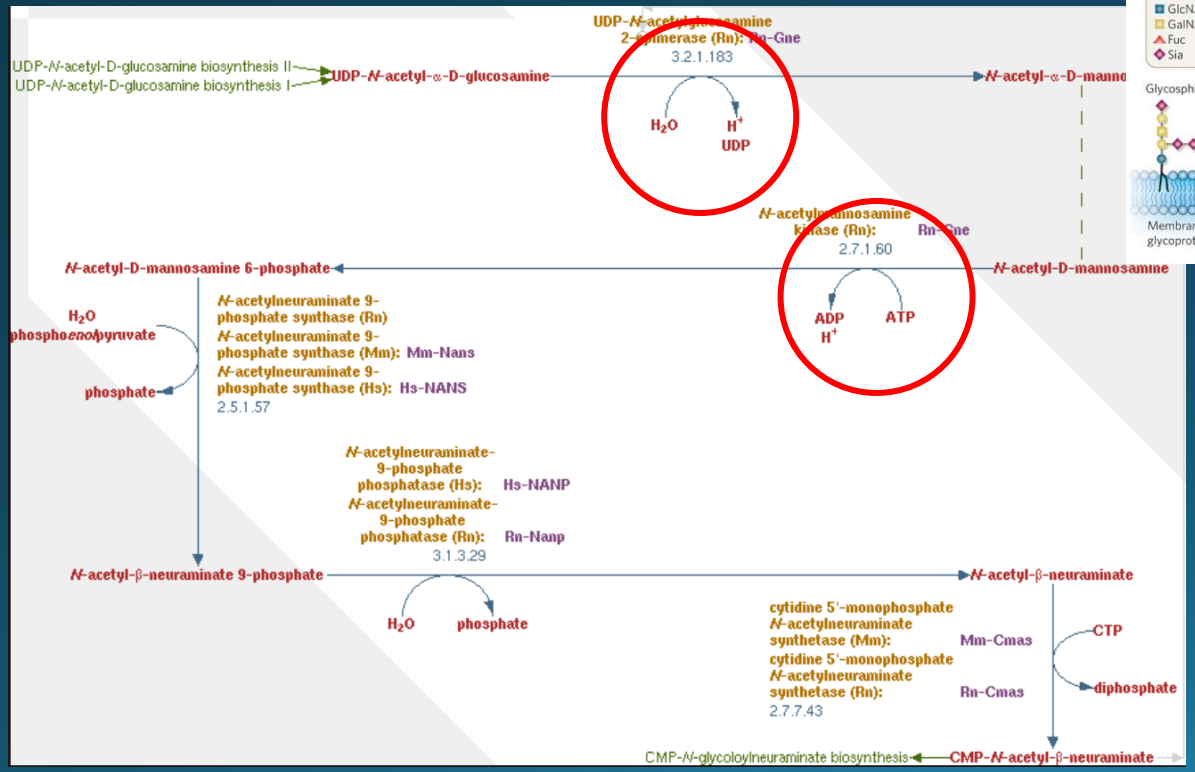
ATP + GTP + adenosine -> dADP + H_2O + H^+;

2 ATP + NAD^+ + 2 H_2O + guanosine -> dGDP + NADH + 2 H^+;

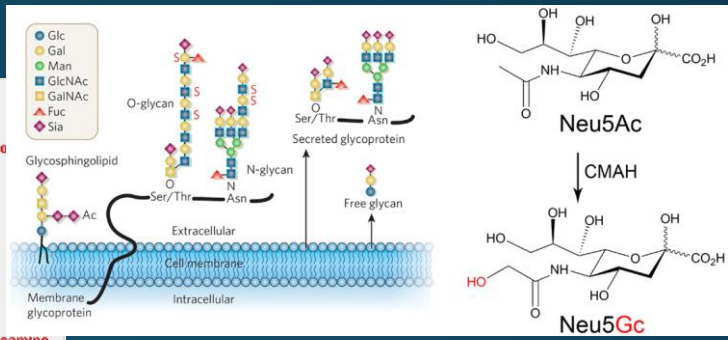
ATP + guanosine -> dGDP + H_2O.

Increased Sialic Acid Production

- All cancers tend to gradually increase their sialic acid and ganglioside synthesis as the disease advances

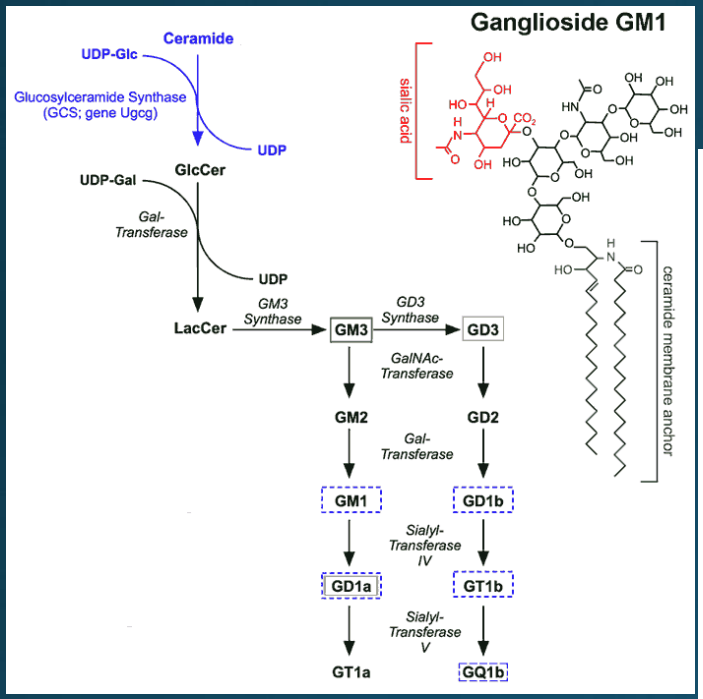


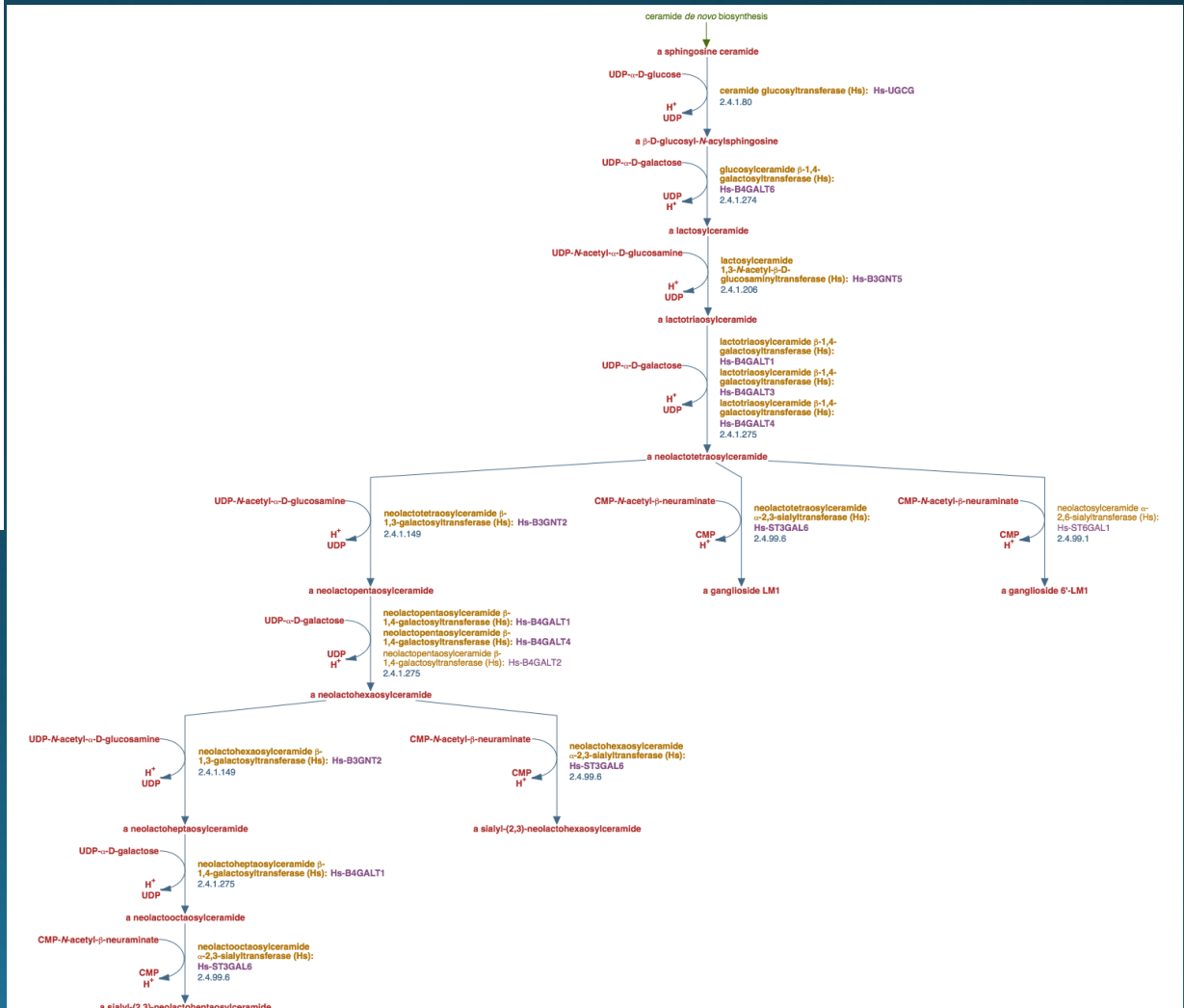
Sialic acid synthesis



Two net protons are produced when a sialic acid is biosynthesized

Increased Ganglioside Synthesis





The synthesis of gangliosides produces numerous protons

Increased Phosphorylation in Cancer

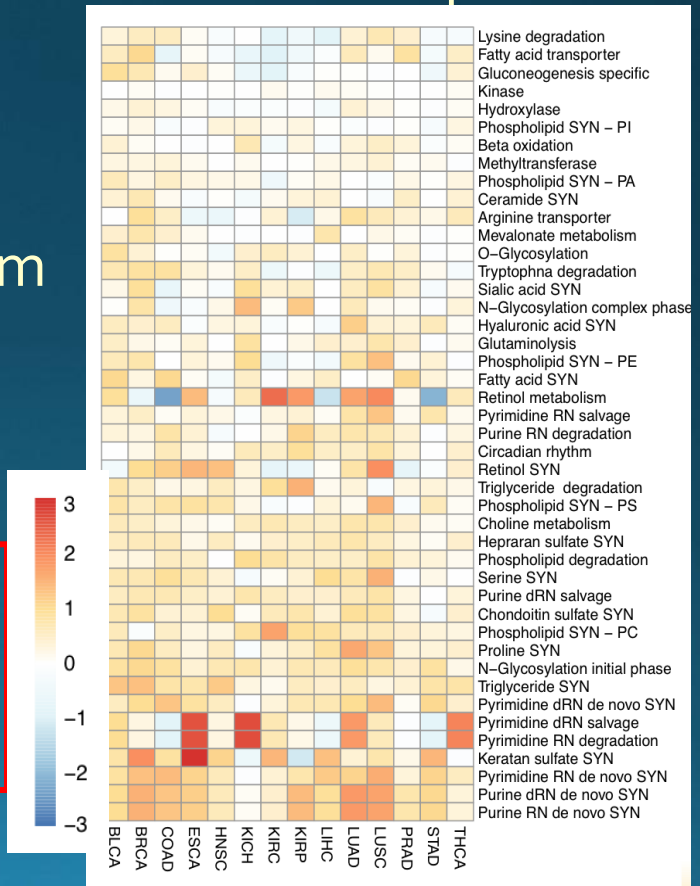
- Cancer cells tend to considerably increase in the activities of kinases, giving rise to increase in phosphorylation

- The process of phosphorylation generally produces one proton for each act as it requires the following ATP hydrolysis reaction

- $ATP \rightarrow ADP + H^+$

Extensive Metabolic Reprogramming in Cancer

- We have analyzed ~50 reprogrammed metabolisms across 14 cancer types, 7,000+ samples in TCGA
- Found that every reprogrammed metabolism examined produces more protons than the original metabolism.
- And yet cancer intracellular pH goes up, according to the literature!

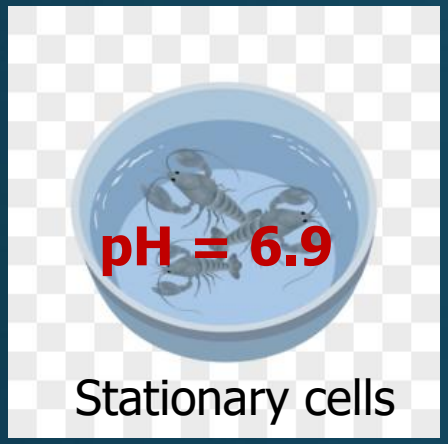


Hypothesis and Our Approach

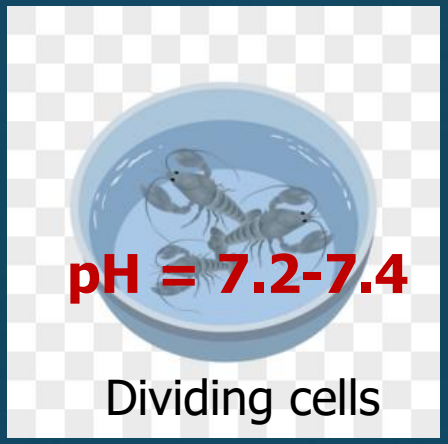
- There must be some unknown processes that continuously produce OH^- or equivalent

- A possible angle could be chronic inflammation as it is known that all cancers are associated with chronic inflammation

A Primer: Cytosolic pH



Stationary cells



Dividing cells

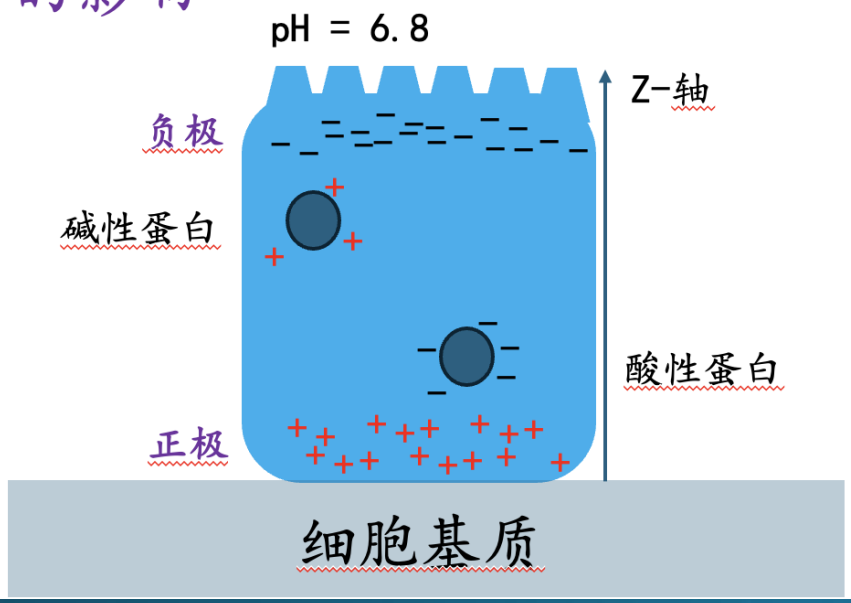
- Normal proliferating cells
- Cancer cells

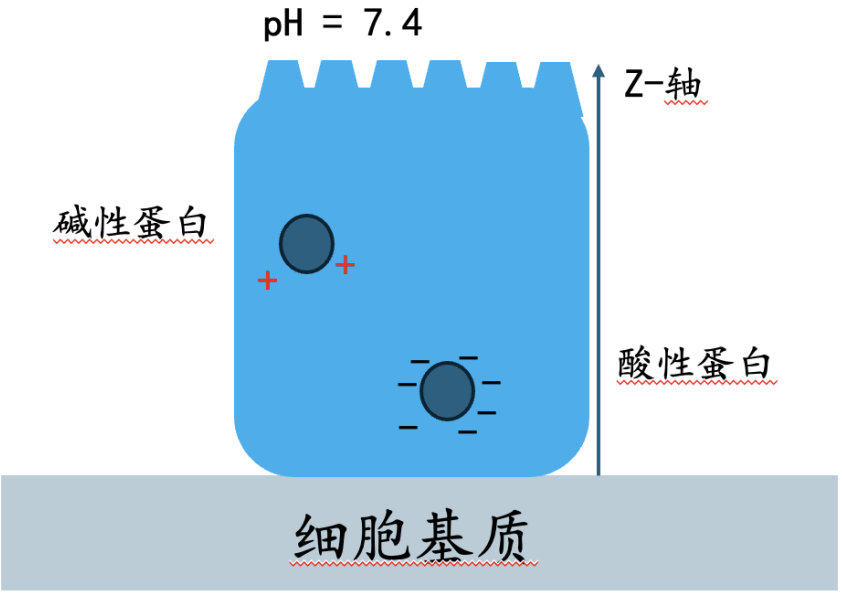
- Human cells, possibly cells of all organisms, use two distinct pH levels in stationary vs. dividing states
- pH dictates protein folding, enzymatic reaction rates, subcellular localization and others
  - E.g., altered pH can lead to protein misfolding such as in AD
- It is vitally important to keep the stability of the pH in both states

Swietach et al., Philosophical Transactions of the Royal Societies, 2014

pH在细胞生物学中的重要性

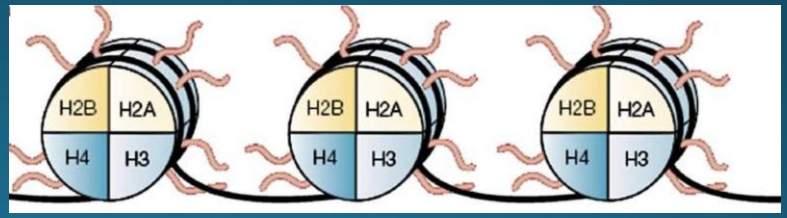
- 人体细胞，如上皮细胞，有一个电场，一端为负电，一端为正电
- 细胞浆的pH帮助带电分子，如蛋白，的空间定位
- pH的改变将影响蛋白沿Z-轴的定位，不同的蛋白可能有不同程度的影响





pH在细胞生物学中的重要性

- DNA带负电、组蛋白带正电，DNA-组蛋白复合体的总电量为0。
- 如果胞浆pH上升，DNA将失去更多H^+,造成DNA的负电量上升；而组蛋白得到H^+的个数将下降，造成其正电量下降。为维持DNA-组蛋白复合体的总电量为0，更多的组蛋白将参与到DNA的packing
- pH在细胞生物学中扮演**极重要的作用**



Maintaining Stability of pH

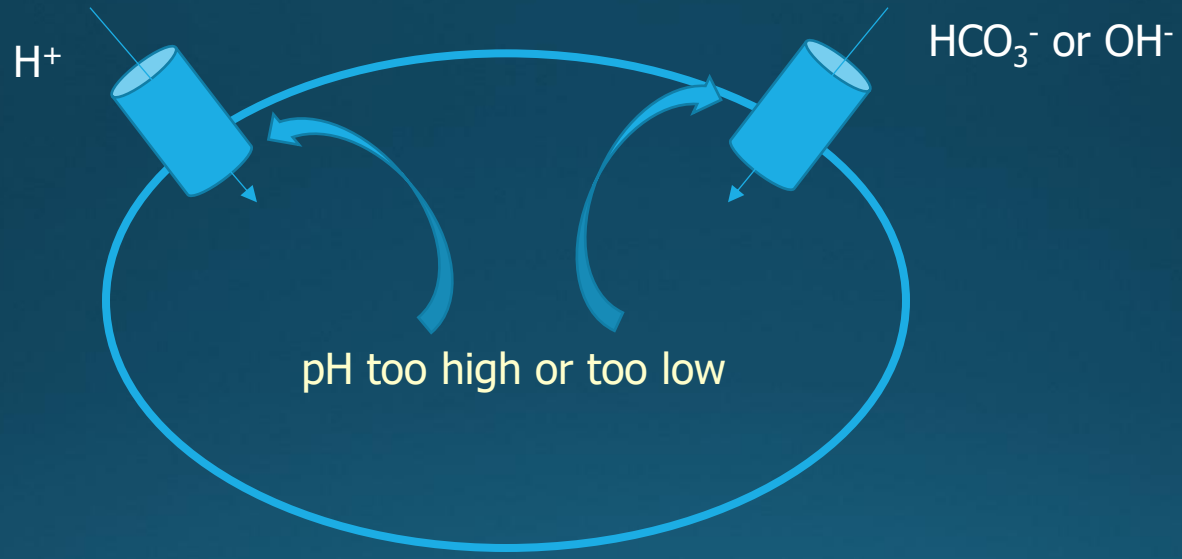
- Human cells have a pH buffer, which can absorb fluctuations in H^+ and OH^- concentrations, and keep the pH stability

$$OH^- + H_2PO_4^- \rightleftharpoons H_2O + HPO_4^{2-}$$
$$H^+ + HPO_4^{2-} \rightleftharpoons H_2PO_4^-$$

- The buffer has a fixed capacity; hence fluctuations in H^+ or OH^- beyond the capacity will alter the pH, leading to acidosis or alkalosis

Proton Pumps

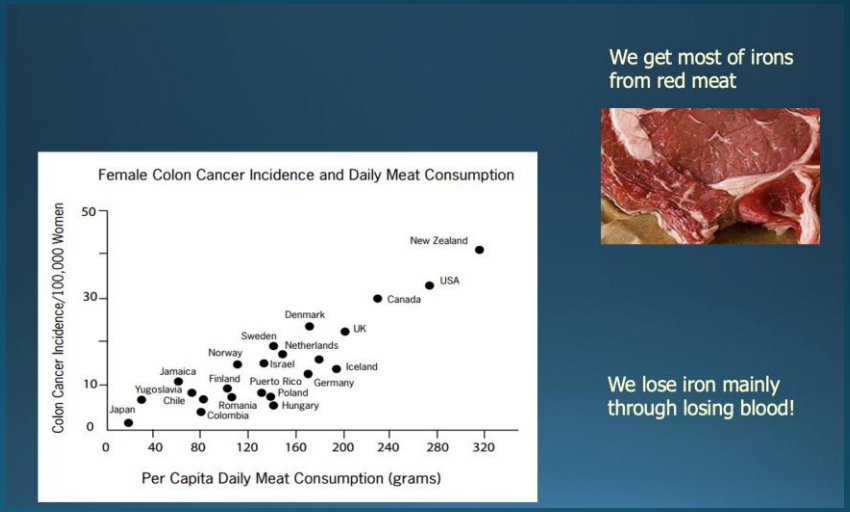
- If the intracellular pH is too high, cells will activate acid-loading transporters to acidify it. Similarly, the pH is too low, cells will activate acid-extruding transporters to alkalinize it



- But proton pumps are not solutions in sustained manner to keep pH neutral while maintaining electric neutrality

Searching for Sources of Alkalinity

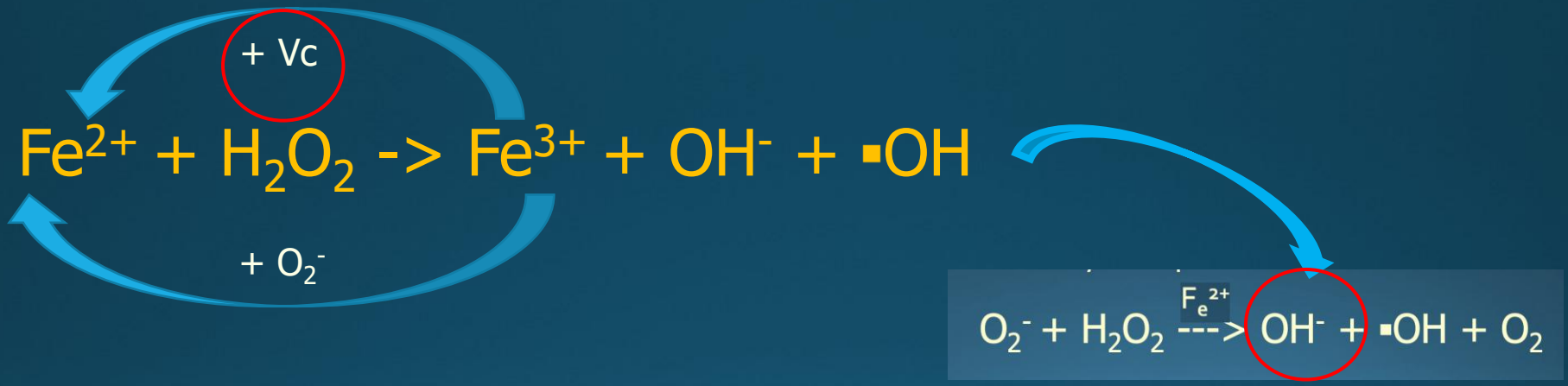
- Chronic inflammation: it is now generally accepted that all cancer is associated with chronic inflammation
- Chemically, the levels of H_2O_2 and $O_2^{\cdot -}$ (released by innate immune cells) are significantly elevated



- Cancer occurrence rates
  - Man vs. woman: 3:2
  - Meat eater vs. vegetarian: 3:1
- **Iron** seems to play a role in disease development, including cancer
  - Iron is predominantly from red meat
  - Once it is inside our body, it will not leave unless losing blood.

Searching for Cause of Acidifying RMs

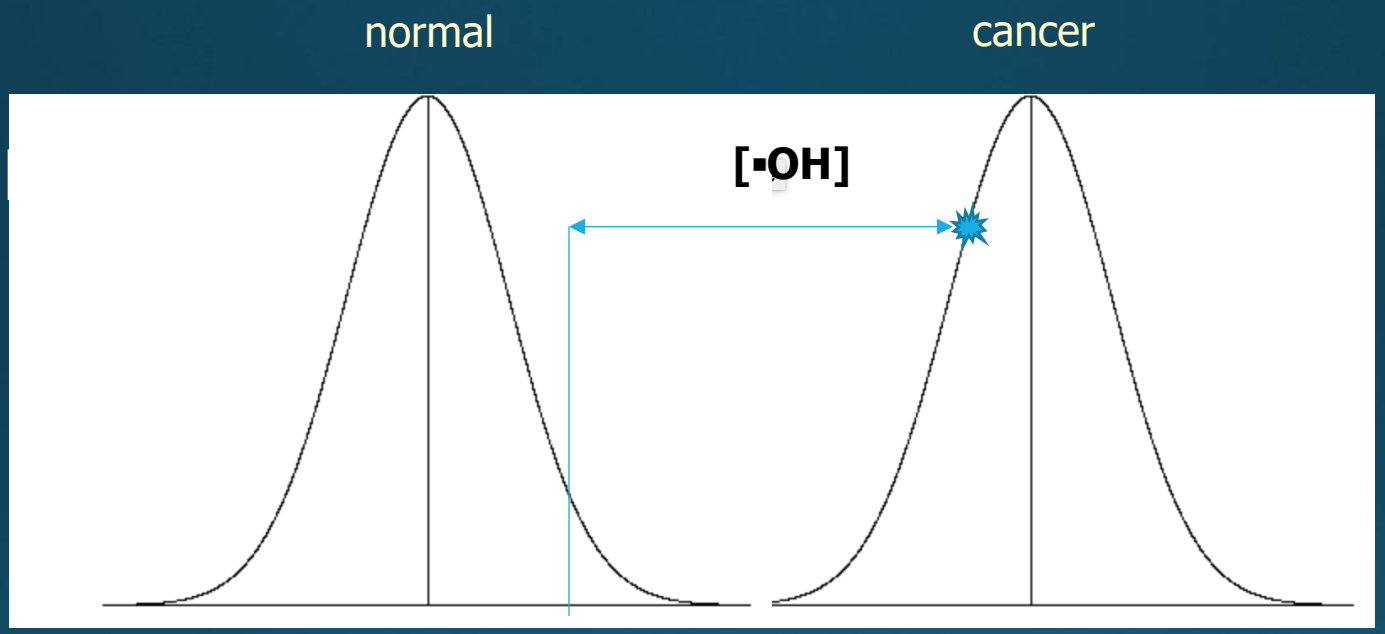
- When chronic inflammation (H_2O_2, O_2^-) meets with iron, Fenton reaction takes place



- We predict: all the MRs are induced to produce protons to keep pH stable when Fenton reactions reach beyond certain levels
- Vitamin C can serve the same purpose of O_2^-

Sun, Zhang, et al, JMCB, 2018

Estimating Relevant Quantities



Distributions of expression levels of proteasome and RNA degradation genes in normal *versus* cancer tissues

Predicting Fenton Reactions

- We use Michaelis-Menten equation to estimate the reaction rate of Fenton reaction

$$\frac{d[\cdot OH]}{dt} = \frac{K_{cat}^{RA}\left(\sum_i a_i X_i^{Fe} + a_0\right)}{\frac{K_1^{\square}}{1} + \frac{K_2^{RA}}{\sum_j b_j X_j^{ROS} + b_0} + \frac{K_3^{RA}}{\sum_k c_k X_k^{RA} + c_0} + \frac{K_4^{RA}}{\left(\sum_j b_j X_j^{ROS} + b_0\right)\left(\sum_k c_k X_k^{RA} + c_0\right)}}$$

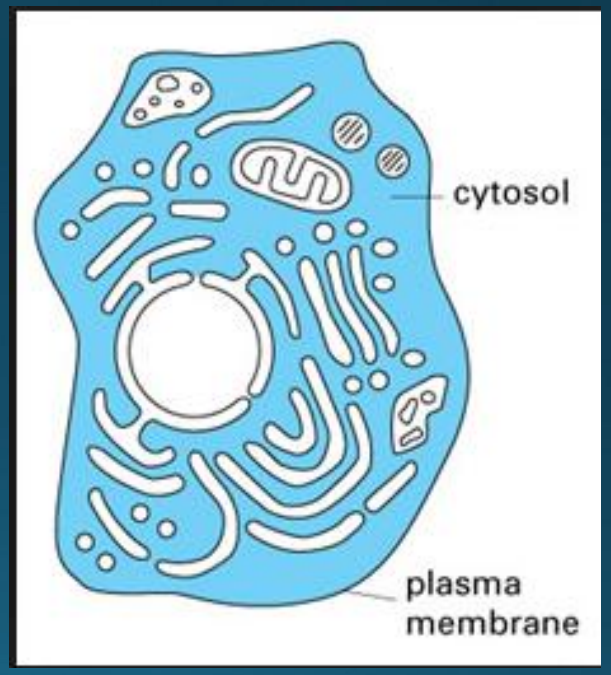
$$+ \frac{K_{cat}^{O_2^-}\left(\sum_i a_i X_i^{Fe} + a_0\right)}{\frac{K_5^{\square}}{1} + \frac{K_6^{O_2^-}}{\sum_j b_j X_j^{ROS} + b_0} + \frac{K_7^{O_2^-}}{\sum_m d_m X_m^{O_2^-} + d_0} + \frac{K_8^{O_2^-}}{\left(\sum_j b_j X_j^{ROS} + b_0\right)\left(\sum_m d_m X_m^{O_2^-} + d_0\right)}}$$

$$+ \sum_n e_n X_n^{\text{Iron Accumulation}} + e_0 + \varepsilon$$

Cytosolic Fenton Reactions

- We predicted that all cancers have Fenton reactions in cytosols (a cellular compartment).

Cancer types	Significance of up regulated proteasome genes	Significance of up regulated mRNA degradation genes	Averaged R^2 value	Significance of the M-M model
BLCA	4.94E-07	5.96E-06	0.697	0.000117
BRCA	1.12E-07	0.00828	0.756	0.000505
COAD	4.86E-07	8.46E-14	0.783	3.75E-05
ESCA	1.02E-19	2.92E-17	0.709	0.00144
HNSC	3.00E-04	4.92E-06	0.702	0.00145
KICH	2.20E-07	0.0376	0.814	0.00844
KIRC	0.0794	0.741	0.852	7.50E-05
KIRP	3.28E-05	0.292	0.842	2.01E-06
LIHC	0.0056	7.83E-05	0.761	0.000702
LUAD	8.50E-10	7.74E-07	0.772	0.000431
LUSC	2.42E-09	9.71E-12	0.701	0.0018
PRAD	6.02E-05	0.000717	0.828	8.69E-06
STAD	2.28E-11	1.24E-09	0.774	0.000877
THCA	8.41E-05	1	0.864	5.01E-05



Production Rates of #H^+ ≈ #OH^-?

- We have shown statistically via metabolic flux analyses
- H^+ production rate by all MRs strongly correlates with OH^- generation rate by cytosolic Fenton reactions

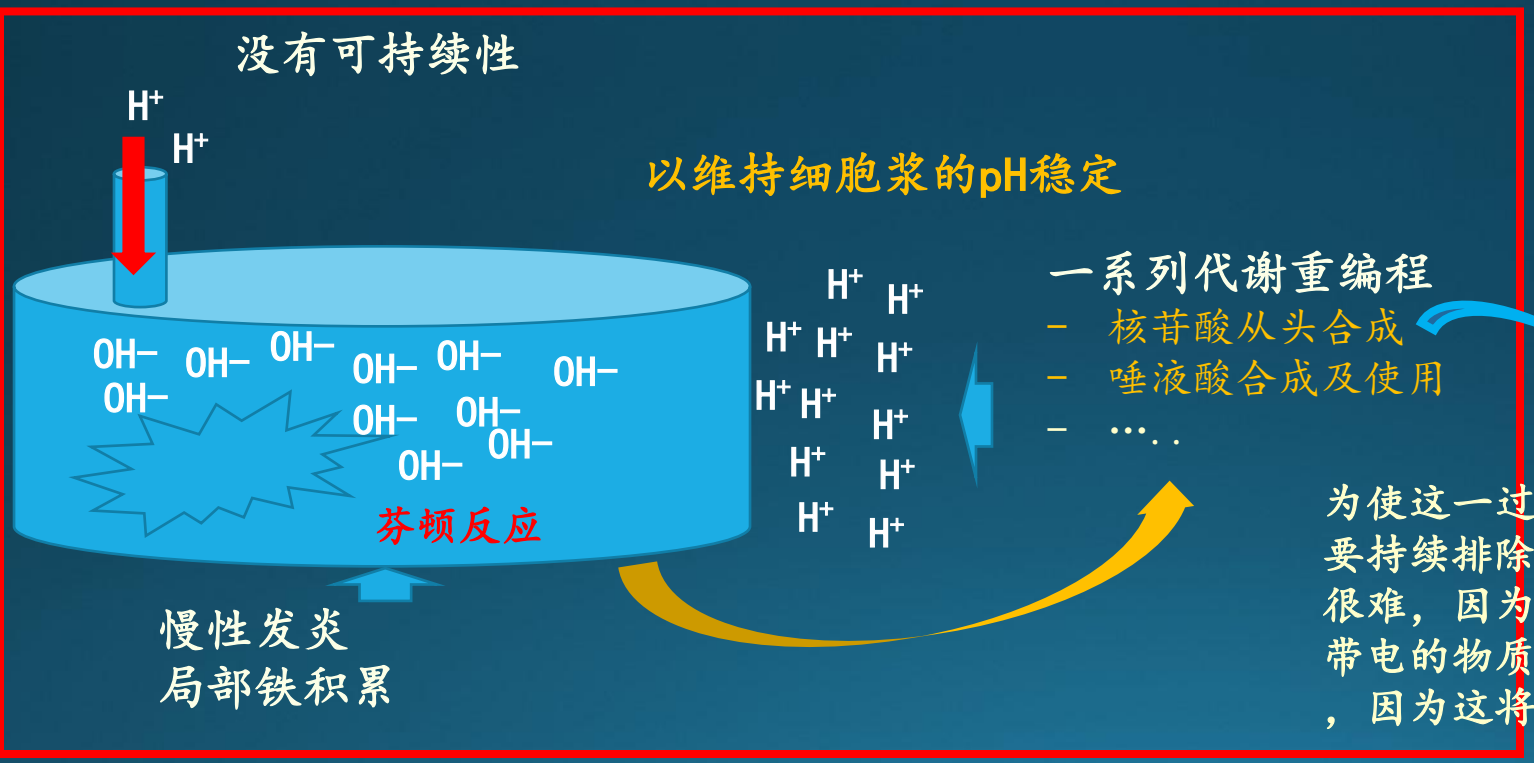
$$\mathbf{R\left(FR,\ OH^{-}\right)} \approx \mathbf{\sum_i R\left(Mi,\ H^{+}\right)}$$

- With this and additional evidence, we predict all the MRs are induced to produce protons to keep pH stable when Fenton reactions reach beyond certain levels

初始模型

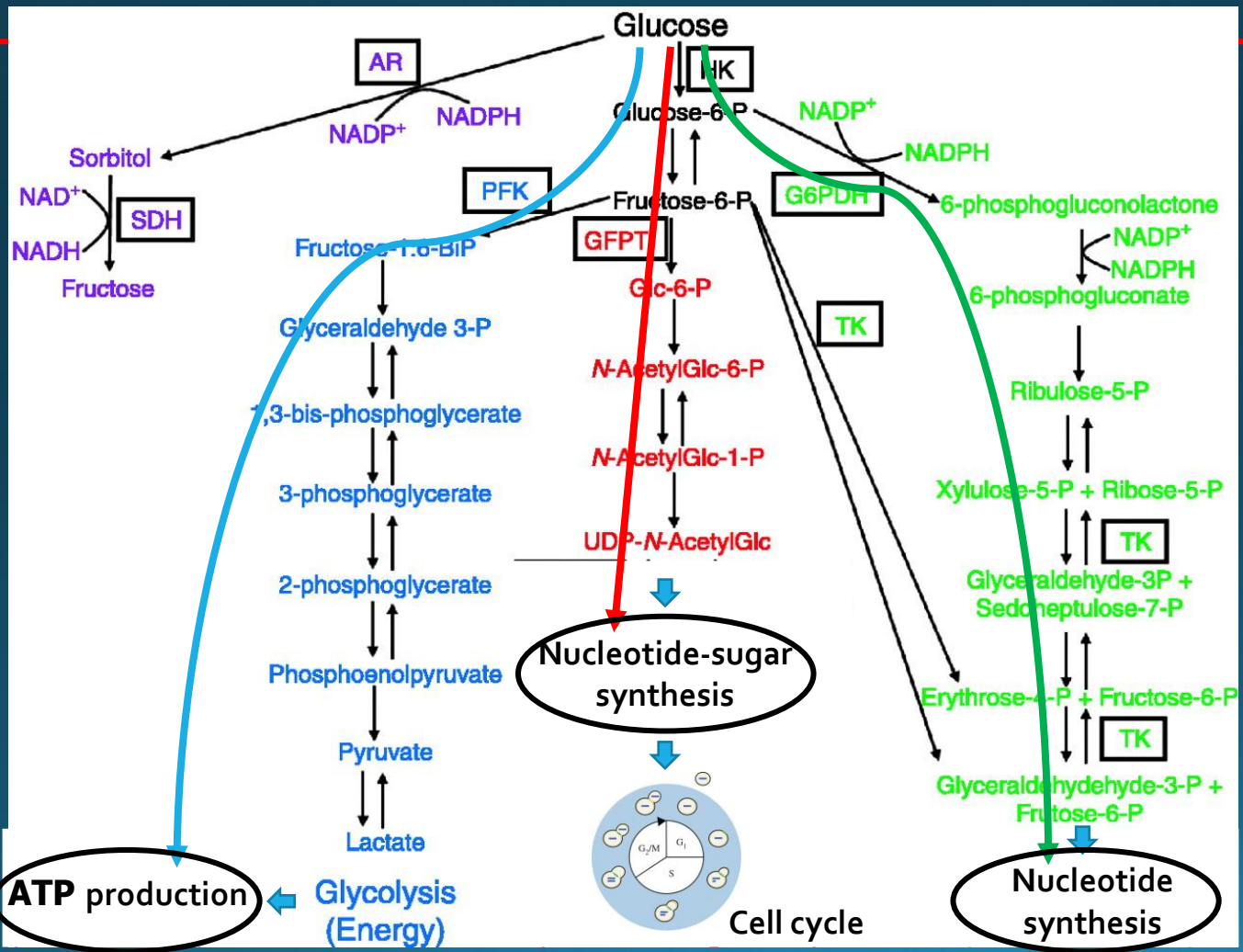
一个大胆的假设：细胞的持续增值是为了将为救命而持续合成的核苷酸快速移出，以保证细胞可以继续不断合成核苷酸

- 细胞 ← DNA^- + 组蛋白$^+$



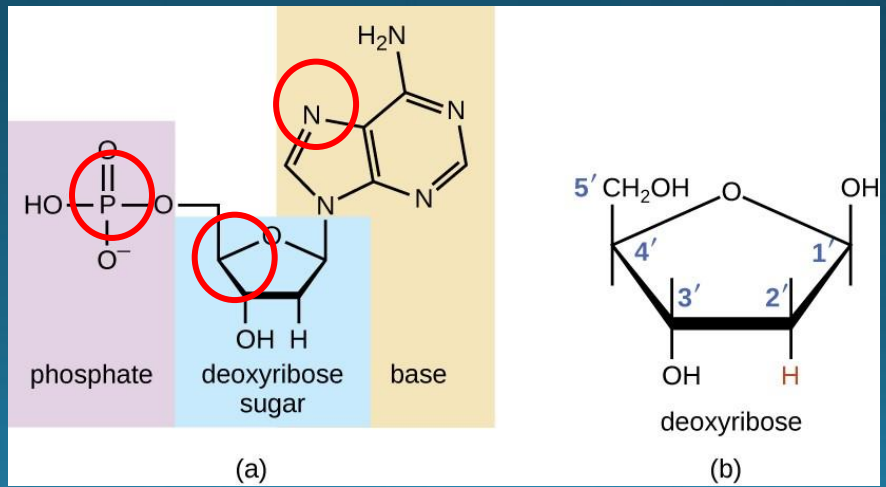
单细胞生物的增殖

只要有食物，单细胞生物会不断吃，首先产生ATP。当其浓度高到一定程度后，细胞转而合成核苷酸；其浓度高到一定程度，细胞开启细胞周期，并合成DNA、蛋白、脂、糖，形成一个细胞，即核苷酸浓度推动细胞周期



Cell Division of Unicellular Organisms

- It is considered that the concentration of UDP-sugar is the cue for cell cycle initiation in bacteria like E. coli
- The possible reason that UDP-sugar is used is that it consists of carbon, nitrogen and phosphate, i.e., when all the essential elements have adequately high concentrations for cell division



Hypothesis

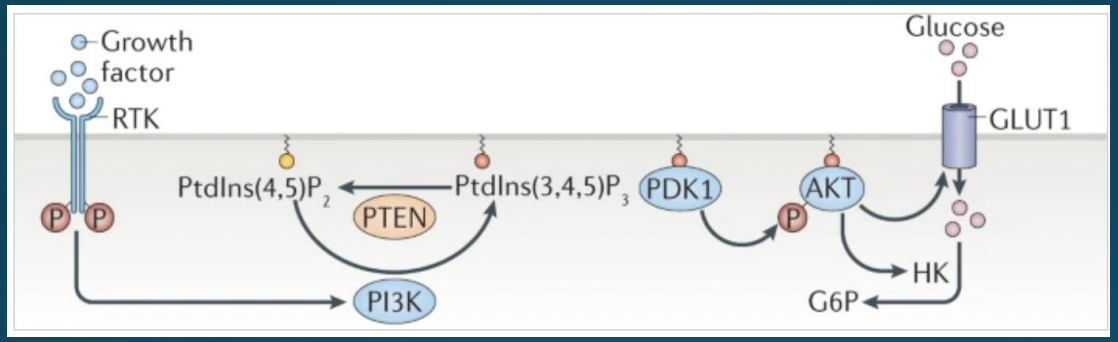
- Is it possible that cancer cells utilize a program similar to the cell division program driven by concentration of nucleotide-sugar, as way to remove rapidly synthesized nucleotides?

- One issue is that human cell division is not driven by accumulation of nucleotides or variants

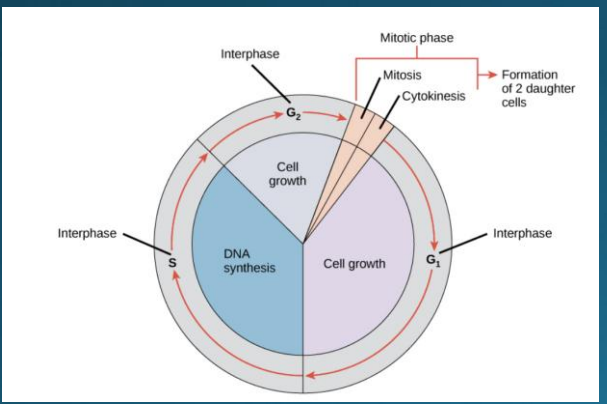
- Question: what changes do human cells need to have, to make such cell division possible?

Cell Division in Human

- Cell division is a top-down process, which is initiated by growth signals and coordinated among cell cycle progression, nutrient balance, cell size, and redox homeostasis



- PI3K/PTEN are the key regulator of cell cycle

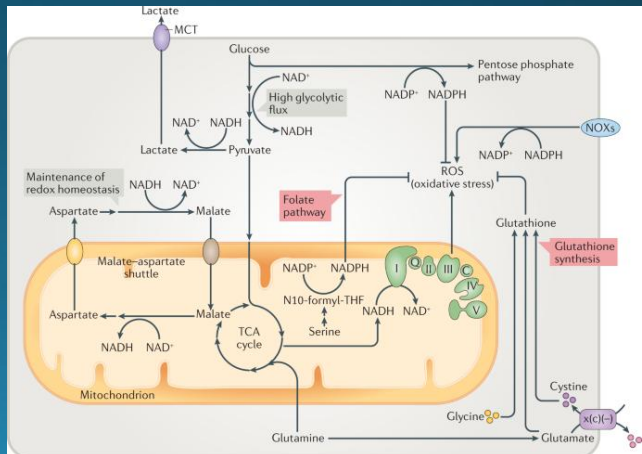


Nutrients

- Glucose
- Amino acids
- Lipids
- Nucleotides

Cell size

Redox homeostasis



Cell Polarity

- Cell polarity provides the supporting infrastructure to functions related to localization, transportation, organization of molecular machinery
  - E.g., protein localization, transport (import, export, secretion, endocytosis, exocytosis), placement of organelles
  - Cytoskeletal structures
  - Lysosome and Golgi are centers of endocytosis and exocytosis, respectively

- Cell polarity is dynamic; in different phases of a cell, different functions need to be performed, hence a different type of cell polarity is built
  - Static state, division, differentiation, migration ….
- Conversely, different cell polarities support a different set of functions

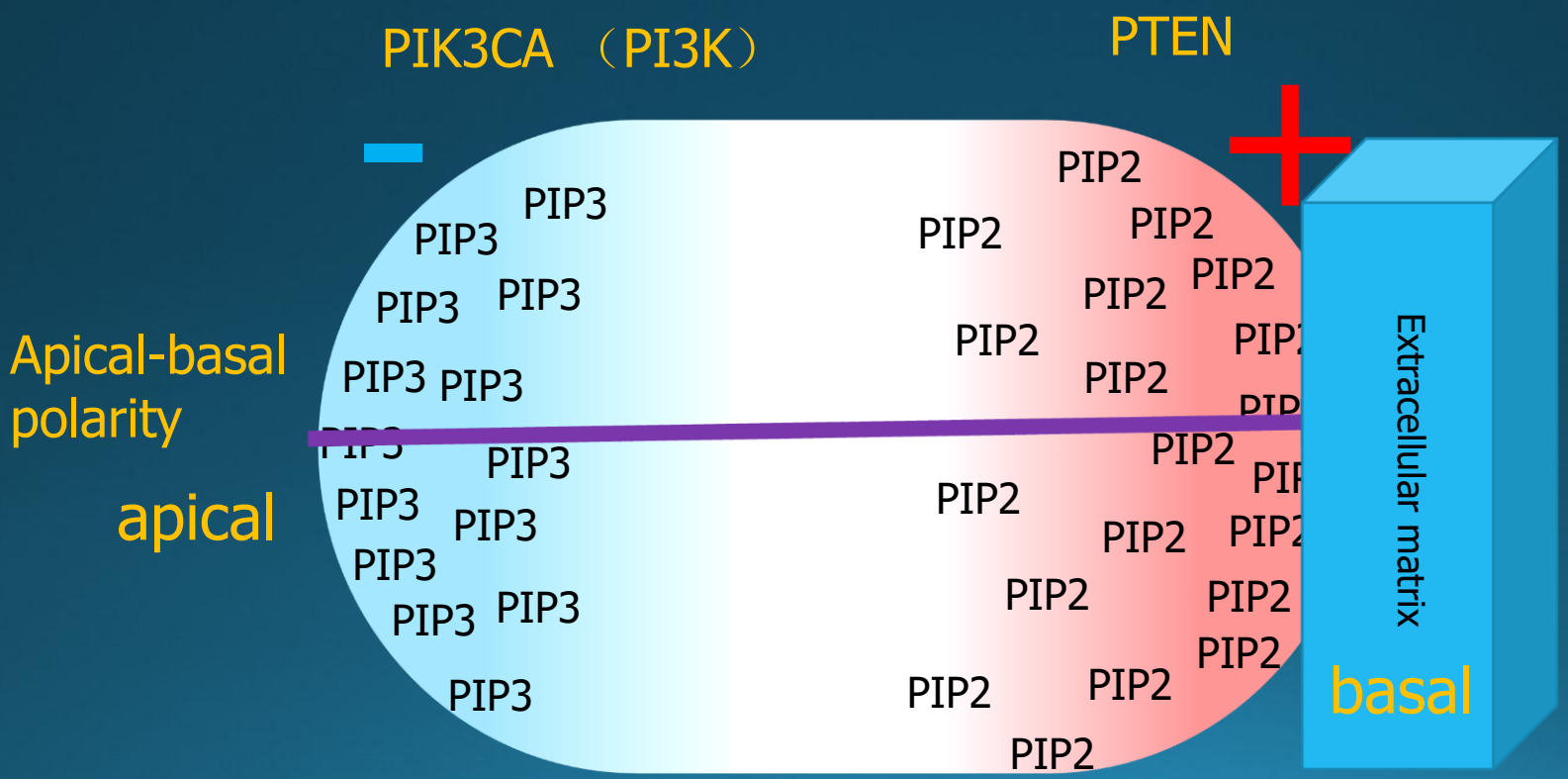
Cell Polarity

- Cells that do complex functions require a sophisticated cell polarity
  - Like freeway, subway, high-speed train system, overpass in a big city like Shenzhen while a village needs only a few roads to support its simple functions

- Cell polarity defines a cell type!

Human Cell Polarity

- In human, the foundation of cell polarity is differential electric charges at the two poles

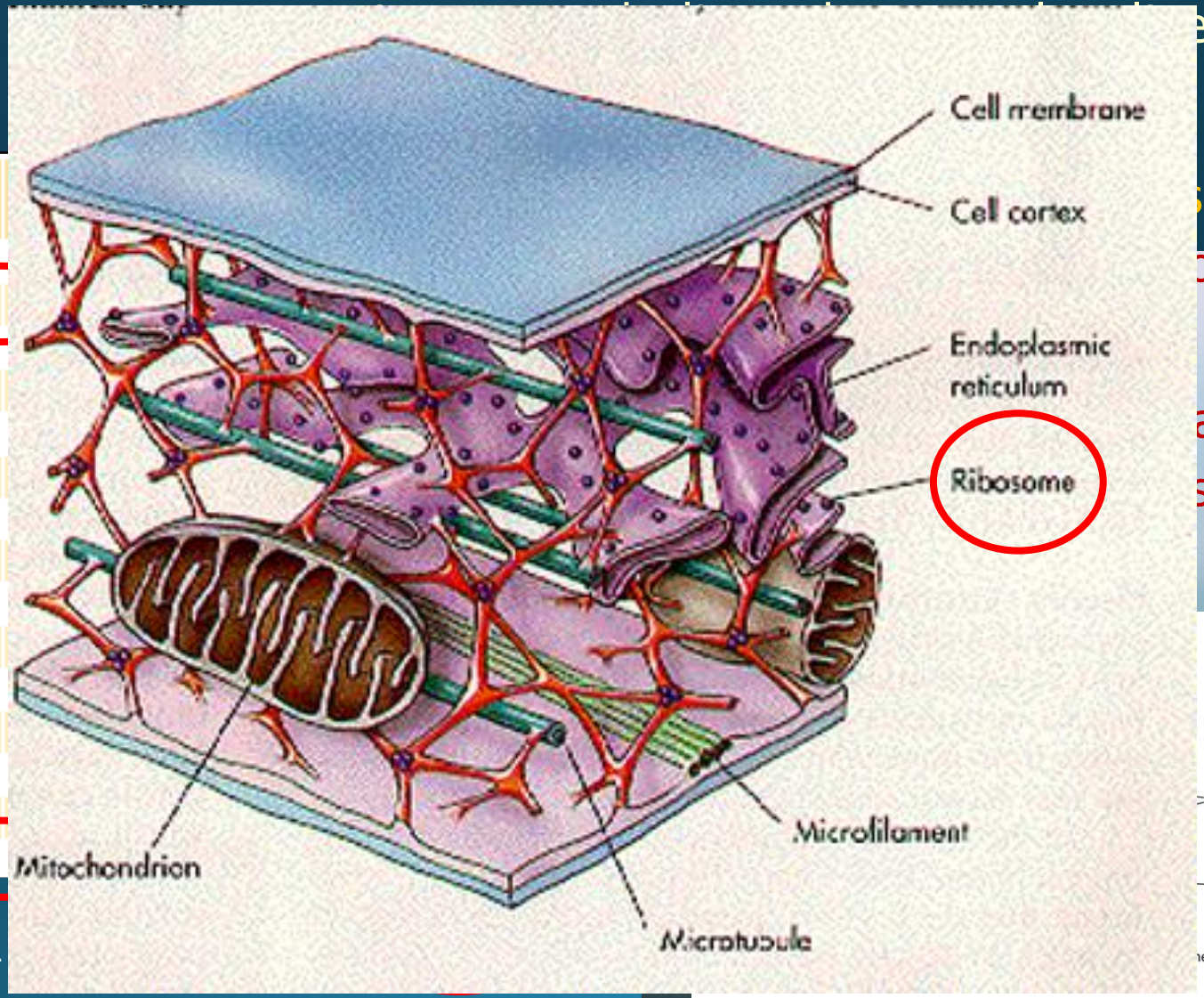


Human Cell Polarity

- How are ed so they can

cell type
sperm cell
red blood cell
lymphocyte
neutrophil
beta cell
enterocyte
fibroblast
HeLa, cervix
hair cell (ear)
osteoblast
alveolar macrophage
cardiomyocyte
megakaryocyte
fat cell
oocyte

$4x10^{13}$: 1



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Plasma membrane

Cytoskeleton

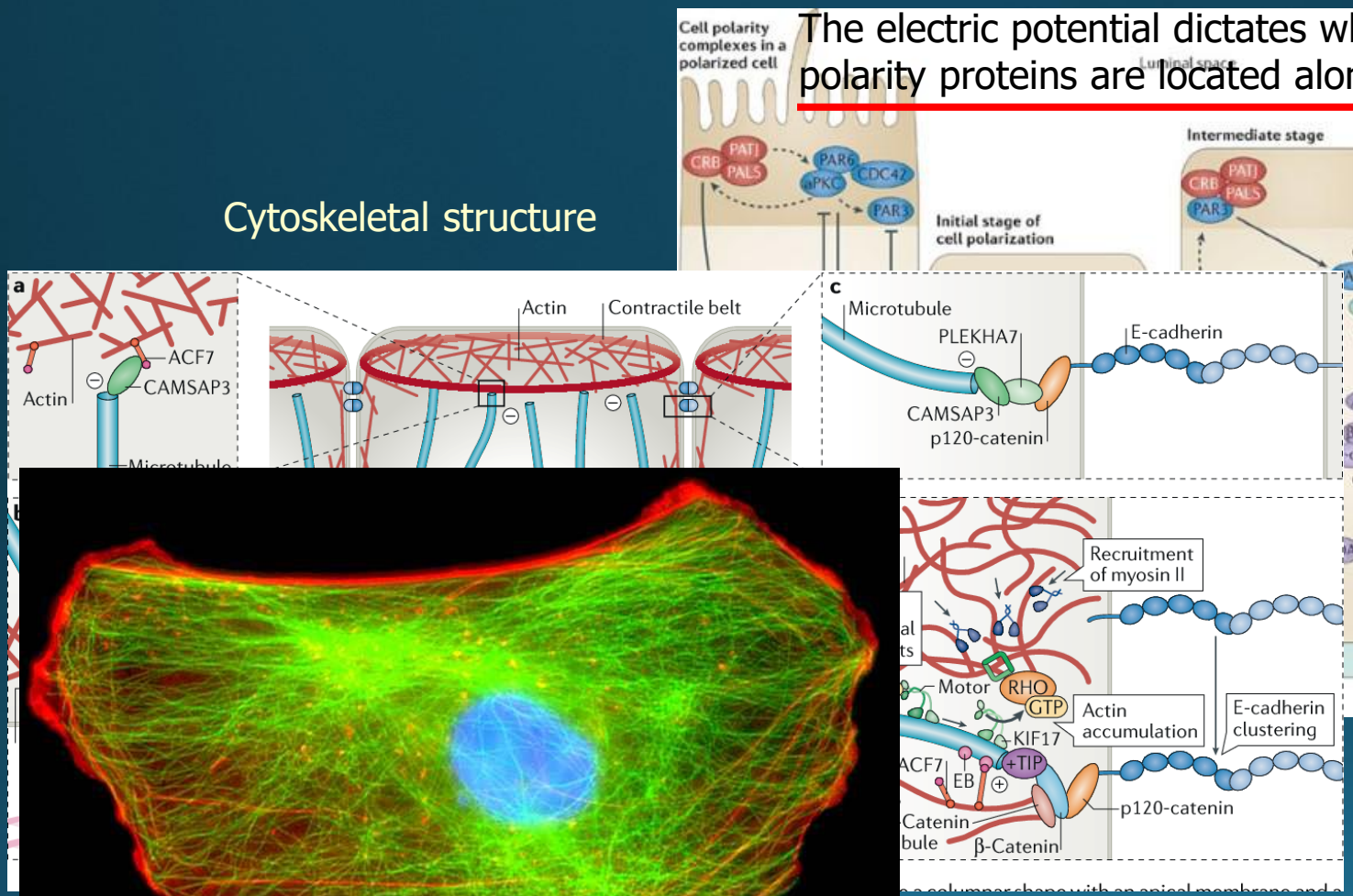
Cytoplasm

Apical-Basal Polarity

The actin filaments and microtubules serve as the infrastructure for localization, transportation and cell shape (along with intermediate filaments), which are regulated by PIPs

The electric potential dictates where the key polarity proteins are located along the main axis

Cytoskeletal structure



Actin filament: red
Microtubule: green

Mutations in Cancer Genome

- We have conducted a systematic analysis of all the mutated genes across all genomes of a cancer type in TCGA

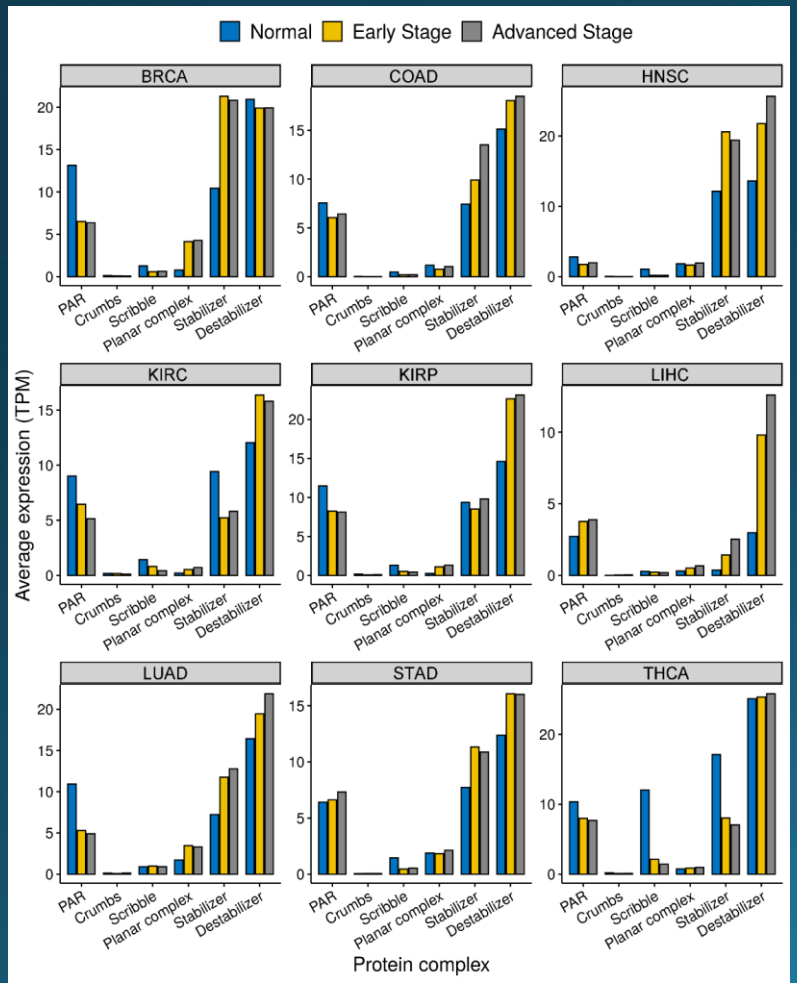
- We found that 40-60% of the mutated genes are part of the polarity system in support of cell division of human cells

Mutations in Polarity Genes in Cancer

- 1. Golgi genes are heavily mutated
- 2. Numerous signaling processes such as WNT, ERBB, AKT, TOR, are highly mutated or/and repressed
- 3. Top ten most mutated genes across different cancers are predominantly involved in cell polarity maintenance
- 4. The enzymes producing PIP3 and PIP2, namely PIK3CA and PTEN, are highly mutated in cancer, particularly PIK3CA representing the most mutated gene across numerous cancer types

Mutations in Polarity Genes in Cancer

- 5. Key polarity proteins are considerably mutated and repressed in cancer



- 6. All cancers express only 3-4 actin genes, ACTB, ACTG1, ACTA2/1, strongly suggesting that the roles of actin are considerably reduced in cancer; similar can be said about microtubule genes, namely TUBB, TUBB4B and TUBA1B; and about intermediate filament genes, namely KRT18-19, KRT8.

Mutations in Polarity Genes in Cancer

- 7. in 4,466 cancer samples of nine cancer types in TCGA, at least 25.15% to 41.02% of all point mutations are related to polarity establishment or maintenance.

- Is it possible that cancer cells may not have any well-defined cell polarity?

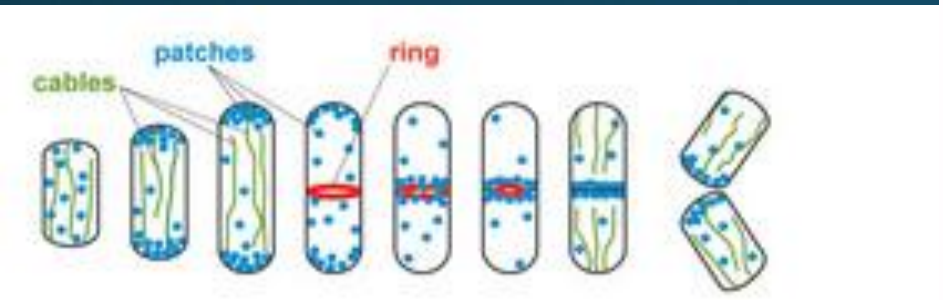
- The answer must be: NO, since cell division is a very complex process, involving the coordination among many different processes consisting of ~1,000 genes.

- Then what does the cell polarity look like in cancer?

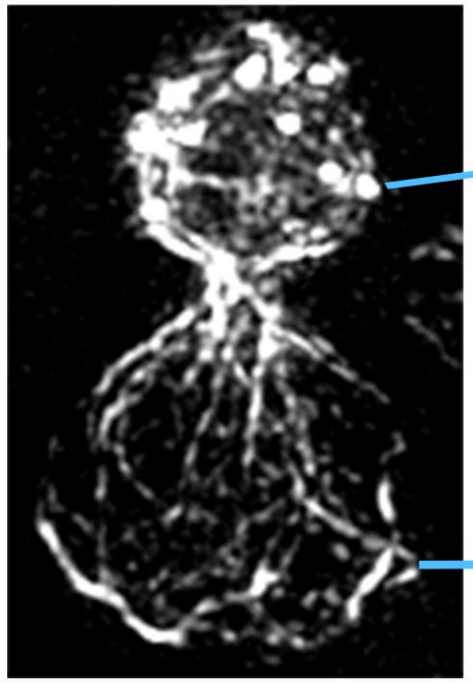
Cell Polarity in Yeast

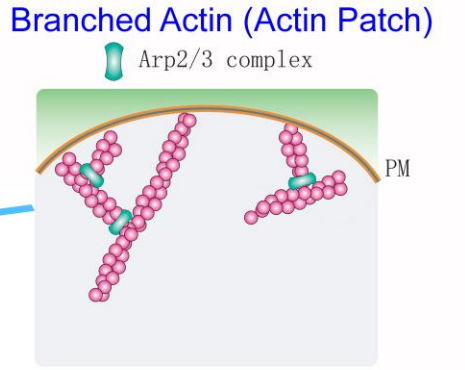
- Yeast has a more complex cell polarity system compared to bacteria

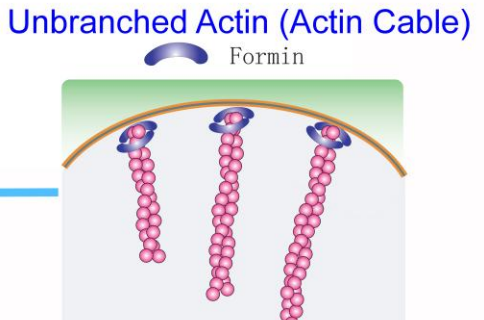
- Actin-like proteins provide the basis for polarity structure for yeast cells



Actin structure	*Nucleation factor*	*Actin organization*	*Myosin motor*
cables:	For3p (formin)	long unbranched bundles	Myo52p (type-V)
rings:	Cdc12p (formin)	short unbranched bundles	Myo2p, Myp2p (-II) Myo51p (-V)
patches:	Arp2/3 complex	branched network	Myo1p (-I)







Cell Polarity in Yeast

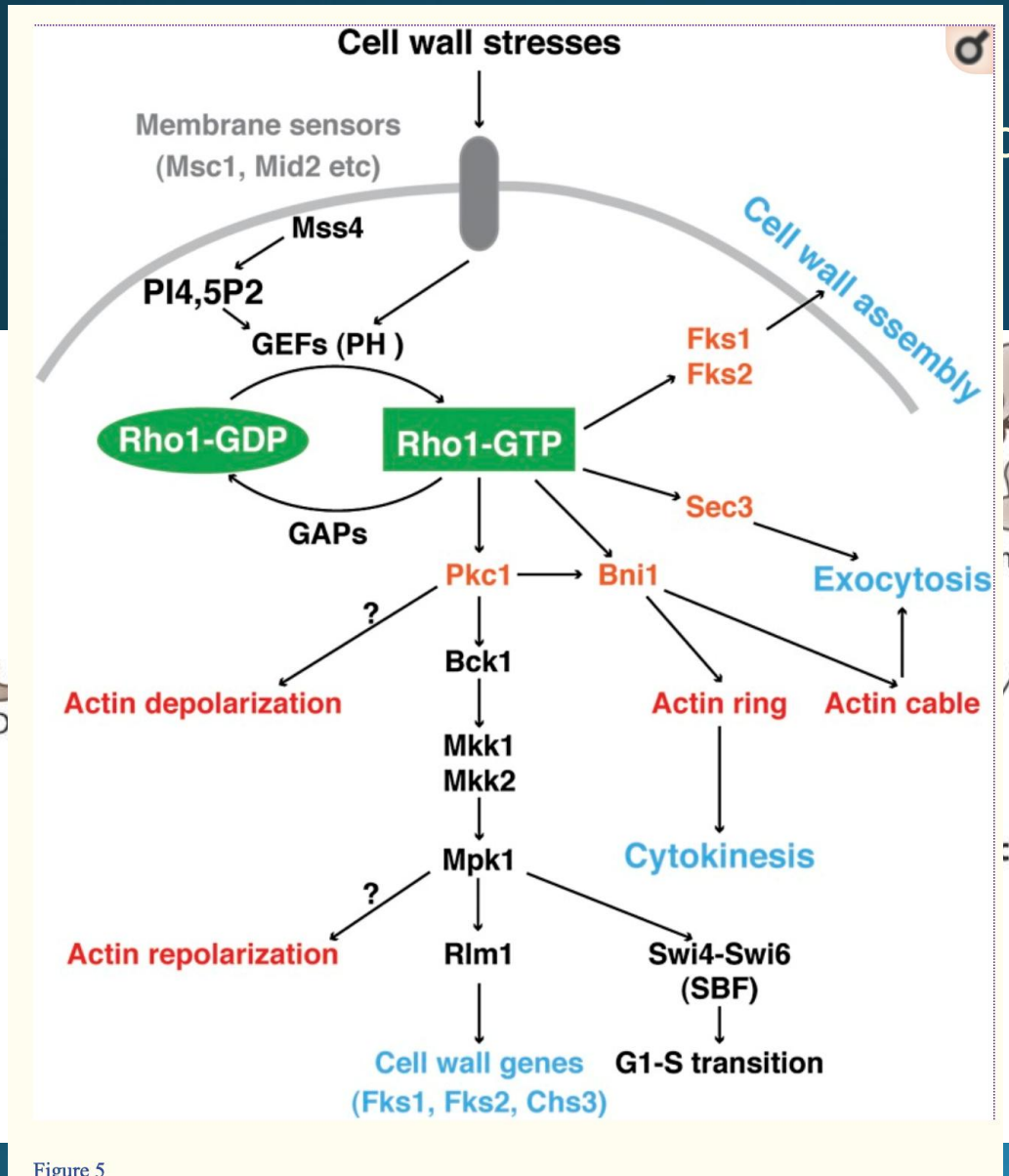


Figure 5

of cell polarity in yeast

regulate interphase actin and microtubule cytoskeleton and symmetric division

cell adhesion and migration

link Rho GTPases to cytoskeleton reorganization and nuclear signaling

vesicular trafficking and the secretory pathway

Cancer's Polarity System

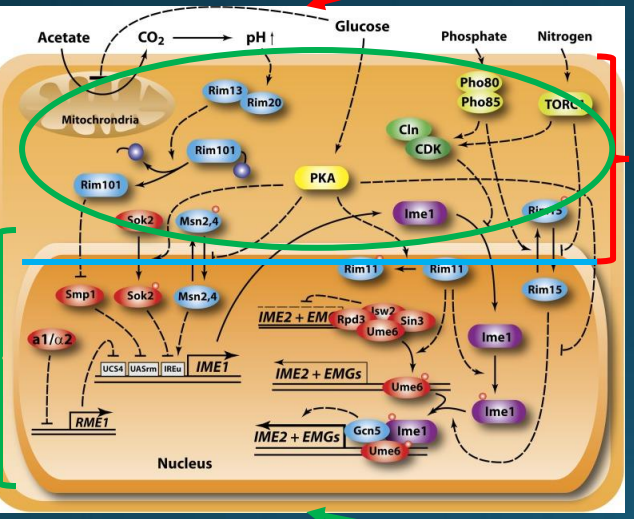
- Mutations to simplify cell polarity, hence cell complexity: at least 50% of all the genomic mutations in a cancer are related to simplification of human's cell polarity machinery to one like in a unicellular organism (X).
  - Many of them have been referred to as "tumor suppressor genes" such as TP53, PIK3CA, APC

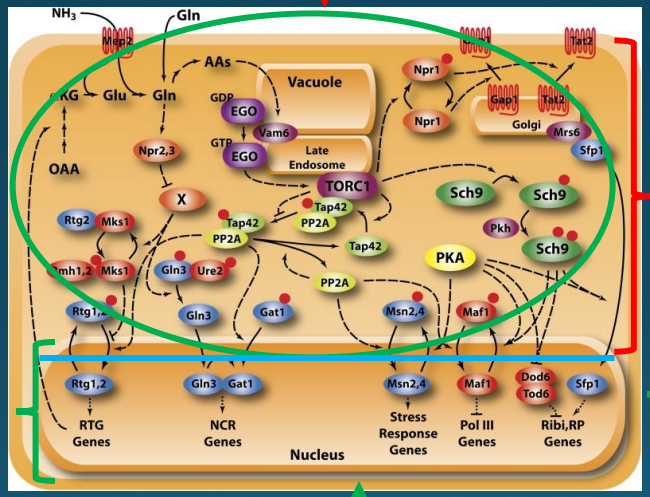
- Our analyses revealed that the set of unmutated polarity genes in support of cell division is similar to that of yeast

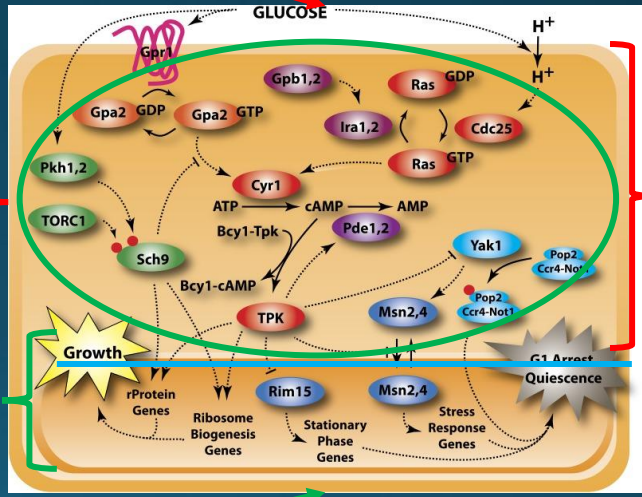
Cell Division of Yeast

Nutrient sensing and related signaling genes

Their human homologs are all oncogenes







Cell cycle program

Yeast cell division is driven by nutrient concentrations

Cancer as a "Unicellular" Organism

- Oncogenes are predominantly nutrient sensing genes in yeast as it has been reported by multiple authors
  - Majority of the proto-oncogenes are from unicellular organisms and key oncogenes such as RAS, MYC, MTOR, are related to nutrient sensing

- We predict that cancer cells are highly simplified cells, like unicellular organisms to enable that nutrient concentrations can drive cell cycle for survival!

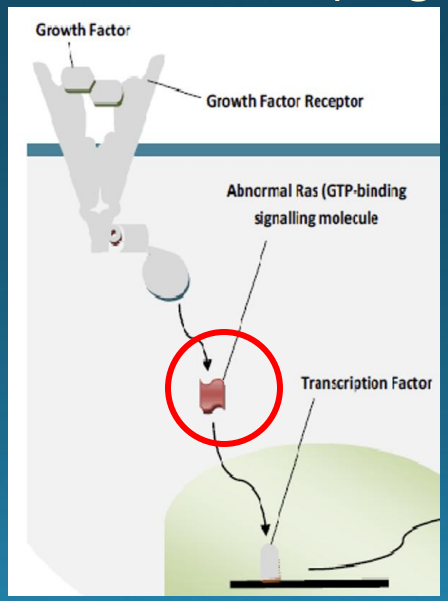
肿瘤中的基因突变

- 我们的分析发现：每个肿瘤组织中～30%的突变发生在用于定义、维护细胞极性的基因
  - 用于细胞内定位、转运、细胞间相对位置的基础设施
- 并进一步发现：肿瘤细胞的极性类似于最早的多细胞生物的极性系统

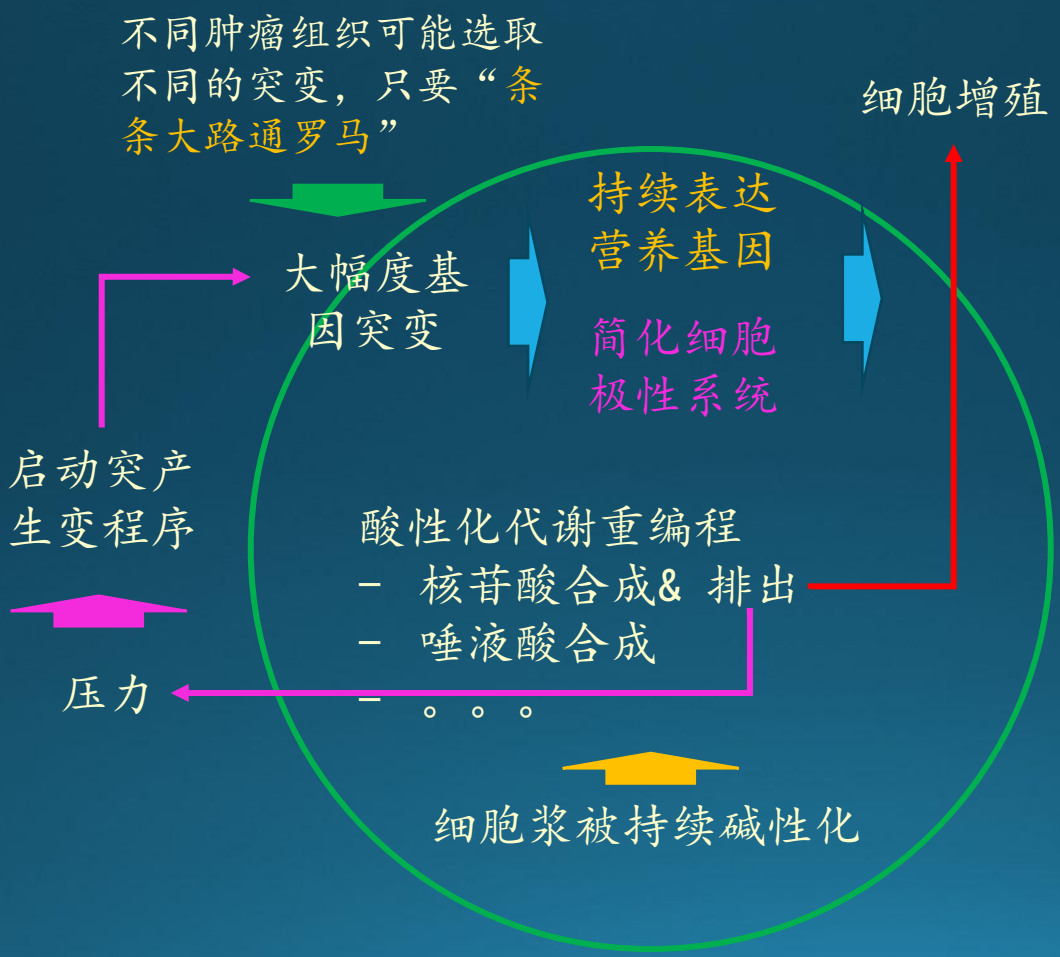
- 新的假设：染上芬顿反应的细胞为了能持续使用核苷酸从头合成来维持pH稳定，大幅简化其细胞结构，使得它能像单细胞生物一样，用核苷酸浓度来推动细胞周期及细胞增殖
  - 支持证据：所有“抑癌基因”都是极性系统的一部分，表明：简化细胞极性是使得细胞由核苷酸浓度推动细胞周期的必要条件
  - 原癌基因都来自细菌，起原始功能多与感知大分子浓度有关，如RAS, MTOR, MYC

Functional Roles of Mutations in Cancer

- Mutate a substantial fraction of cell polarity genes (called tumor suppressor genes in the literature) so to convert a human cell to a unicell-like organism in which nutrient concentrations can drive cell division like a unicellular organism
  - Nutrient concentrations are reflected by the expressions of nutrient-sensing genes or signaling genes, which might be constitutively highly expressed via mutation (oncogenes)



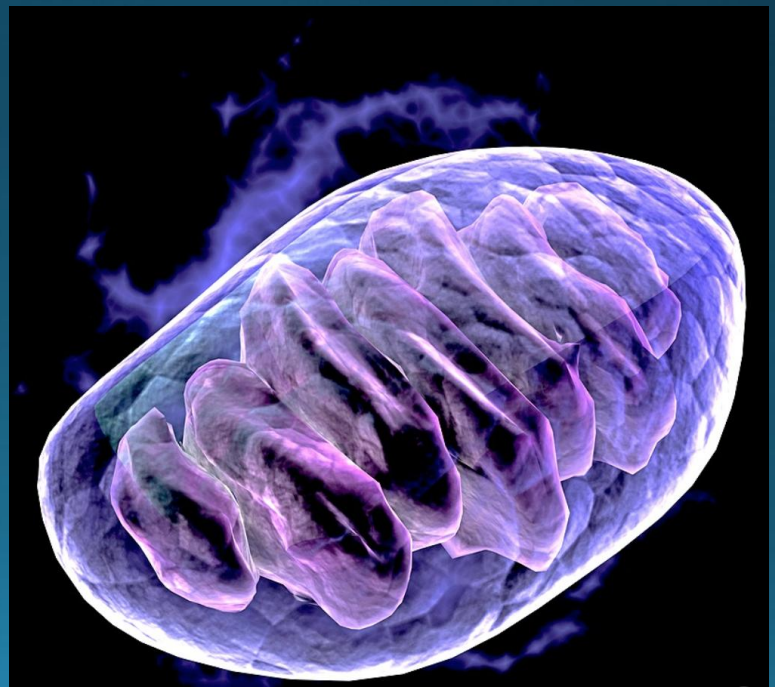
Core Cancer Model



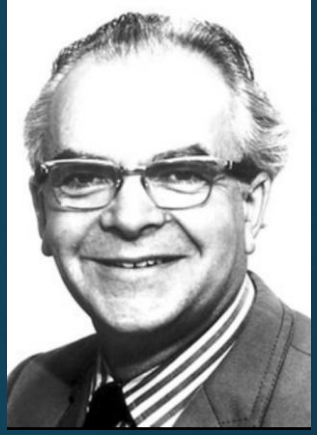
Mitochondrial Fenton Reactions

- Cancer utilizes considerably more ATPs than normal matching cells. Where do the ATPs come from?
- We predict that all cancers have Fenton reactions in mitochondria.

Cancer	Mitochondria		
	P-value of up regulated damage response	Averaged R^2 value	P-value of obtained R^2 value
BLCA	0.91896	0.81373	0.381
BRCA	0.00024	0.82401	0.215
COAD	<1e-5	0.83769	0.076
ESCA	0.65235	0.83374	0.1
HNSC	0.00178	0.84992	0.033
KICH	<1e-5	0.98533	<1e-5
KIRC	<1e-5	0.91672	<1e-5
KIRP	<1e-5	0.91075	<1e-5
LIHC	1	0.90053	<1e-5
LUAD	0.05117	0.85152	0.03
LUSC	<1e-5	0.83877	0.066
PRAD	0.00621	0.92561	<1e-5
STAD	0.03263	0.85049	0.032
THCA	<1e-5	0.95903	<1e-5



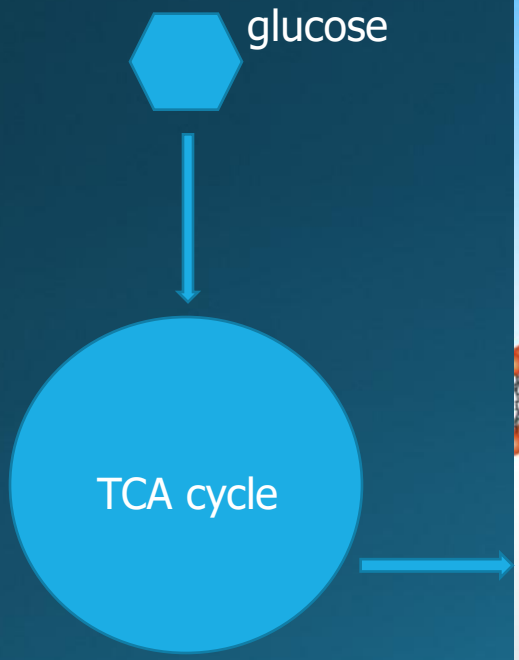
Oxidative Phosphorylation

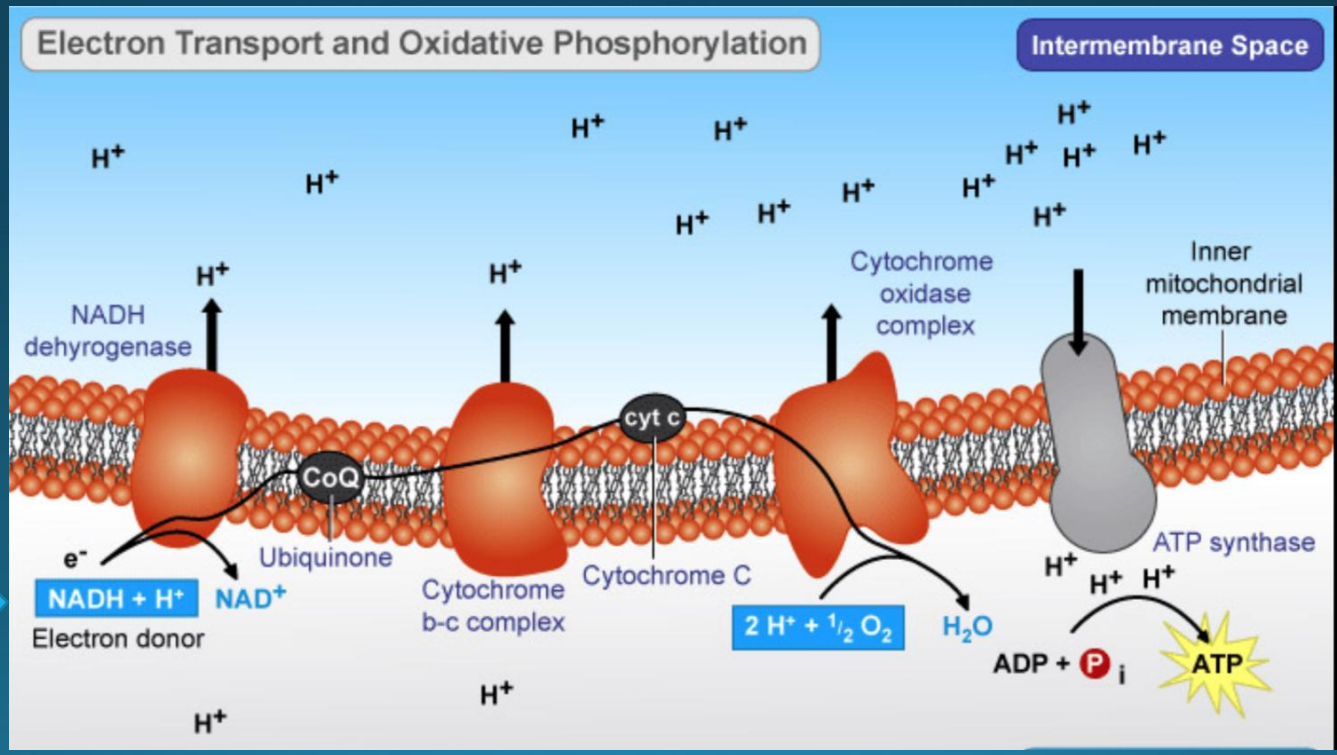


Peter Michell

- Question: where do the OH-'s go inside inner membrane of mitochondria?

- Oxidative phosphorylation: The proton gradient drives ATP synthesis



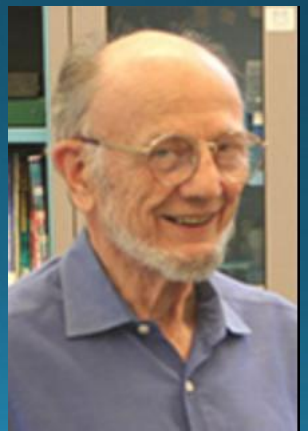


Experimental Validation

- André Jagendorf demonstrated in 1966 that cross-membrane proton gradient can directly drive ATP synthesis by ATP synthase

- … hence experimentally validated Peter Michell's model, and helped Michell to win Nobel Prize in 1978

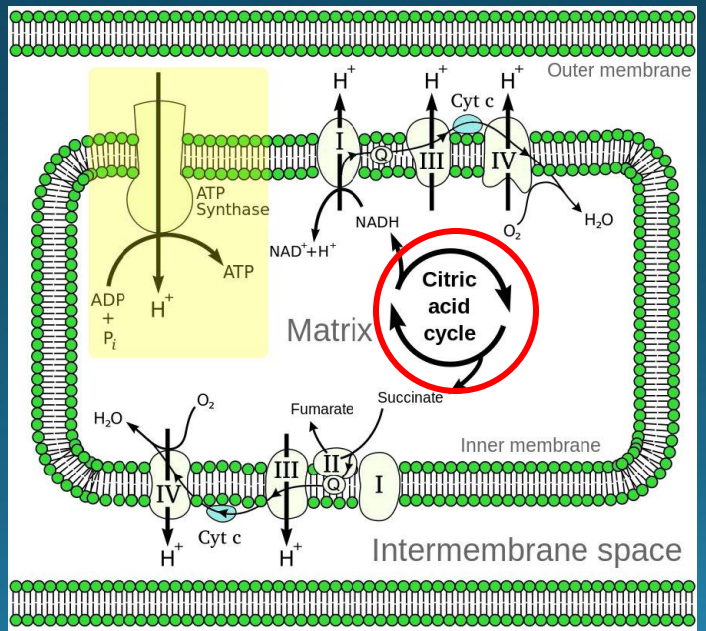
只要水有足够的落差，就能发电

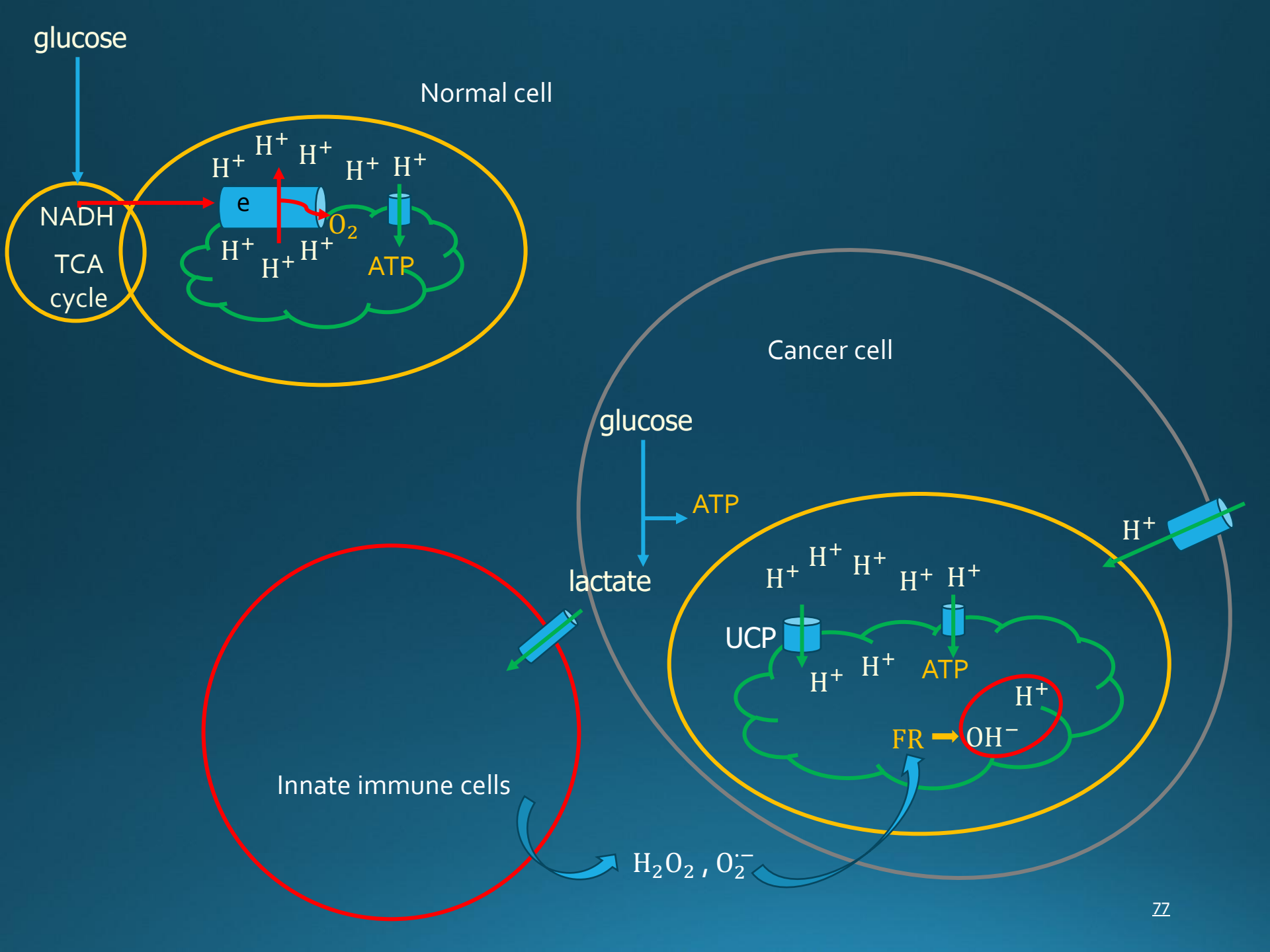


ATP Production by Mitochondria

- It has been established that cancer mitochondria continue to produce ATP through part of the electric transfer chain

- But the TCA cycle is also known to be largely repressed so where the fuel comes from to generate ATP



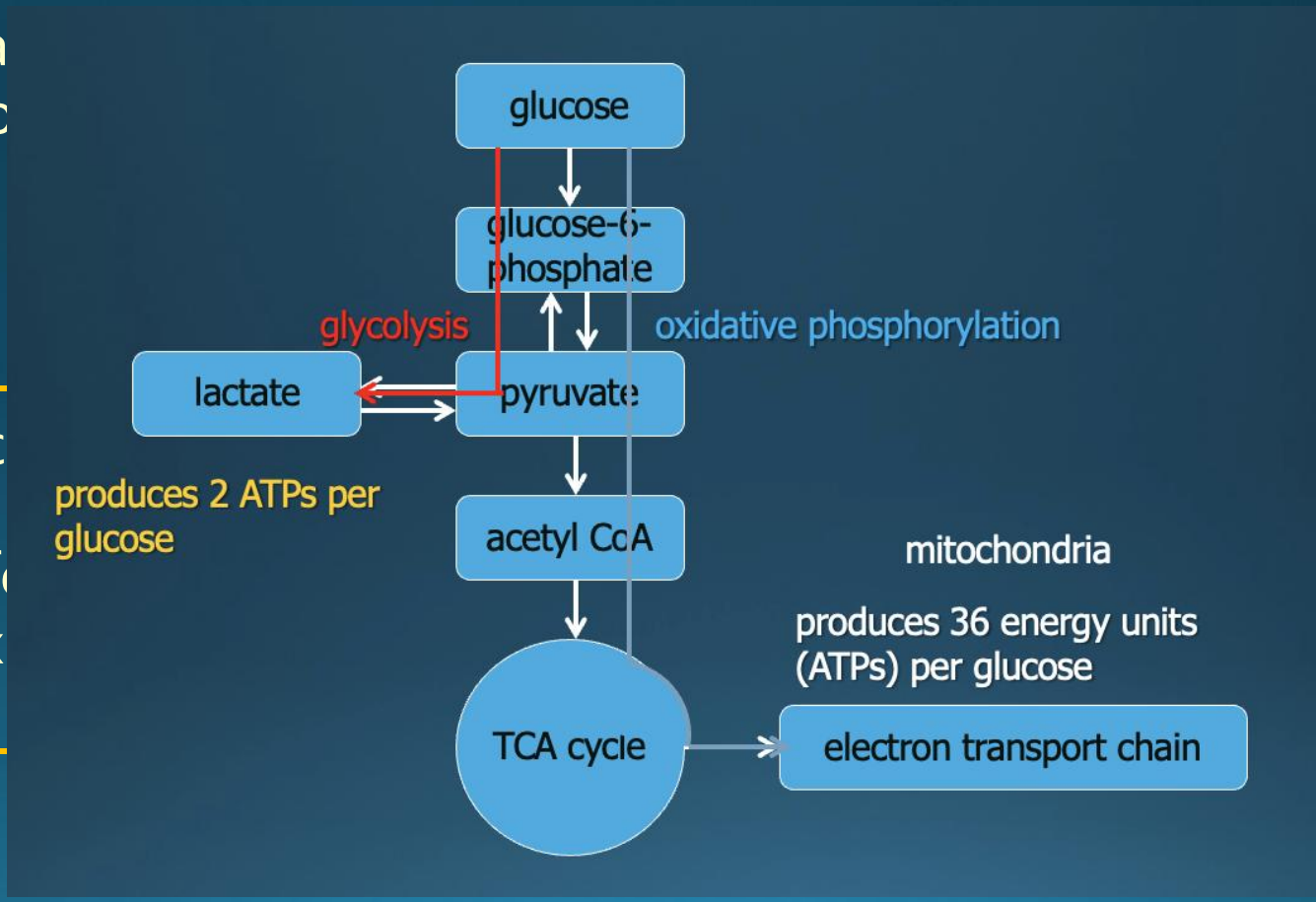

glucose
Normal cell
NADH
TCA cycle
e
O_2
ATP
Cancer cell
glucose
ATP
lactate
UCP
ATP
FR
OH^-
Innate immune cells
H_2O_2 , $O_2^{\cdot-}$

Necessary Conditions of Cancer Occurrence

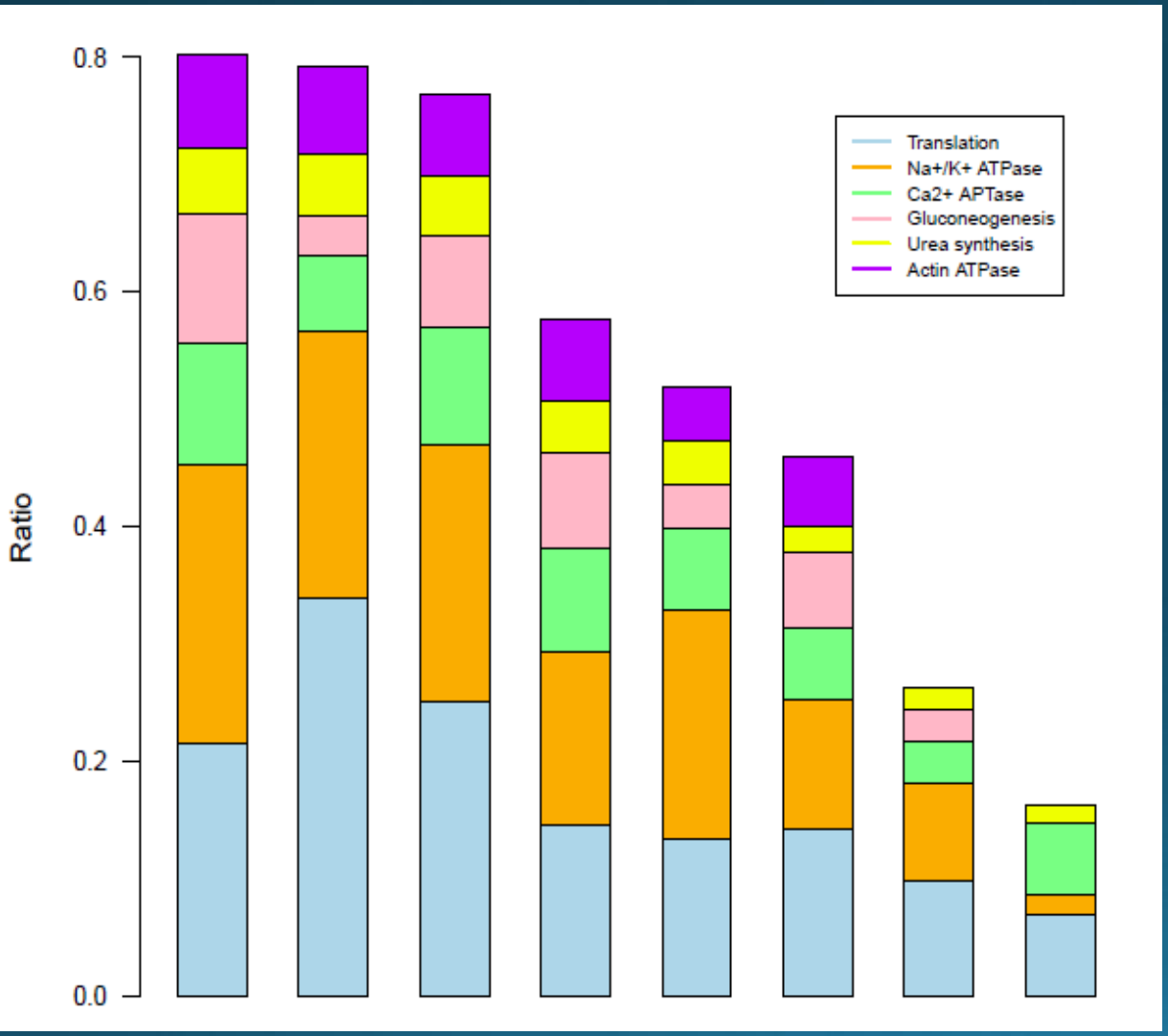
- Why do high levels of mutations do not lead to apoptosis?
- Chronic inflammation has been found to be associated with cancer development, but the mechanism remains not well understood
- Chronic inflammation can be characterized as over-production of H_2O_2 and $\cdot O_2^-$, causing local hypoxia
- Question: what happens when human cells are under hypoxic condition for an extended period of time?

Energy Supply under Hypoxia

- Under hypoxia, human cells switch to (partial) anaerobic fermentation from oxidative respiration for ATP production
- The cells will increa
  reduced energy prc
- Under x% hypoxia,
  accordingly
- This leads to the ac
- The reason is: the f
  deal with the influx



Energy Demand under Hypoxia



- Human, rat and mouse have only little reduction in energy demand under hypoxia *versus* normoxia
- Frog and turtle have significant reductions
- Naked and blind mole rats have substantial reductions

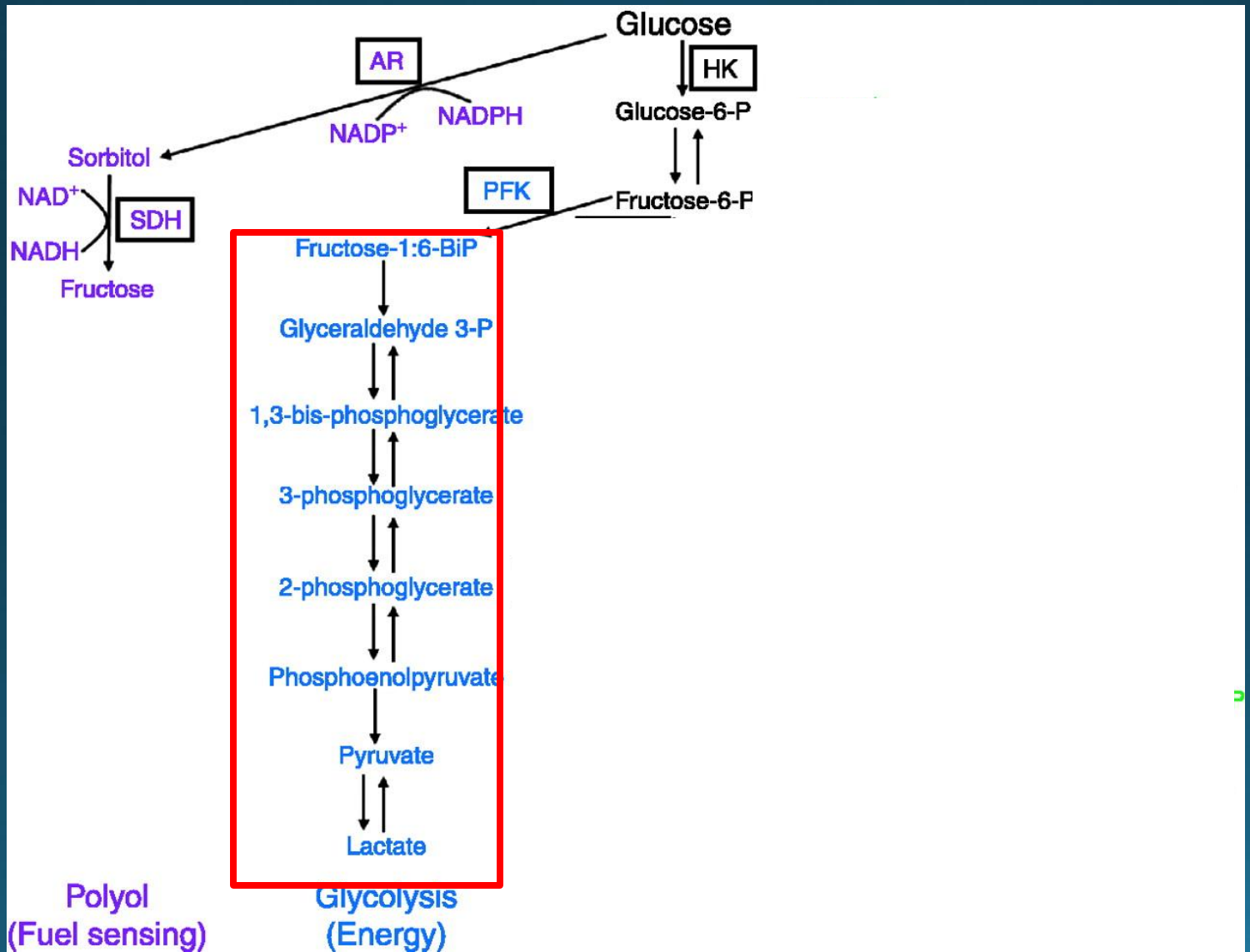
Energy Demand and Supply under Hypoxia

- This is the result of evolution as human has never lived in hypoxic environment for long during the past evolution and hence has not learned which energy-consuming activities to shut down
- Very interestingly, one difference between these two groups of organisms is that one develops cancer and the other virtually do not develop cancers
- Hence the energy gap may have something to do with the fundamental potential of an organism to develop cancer

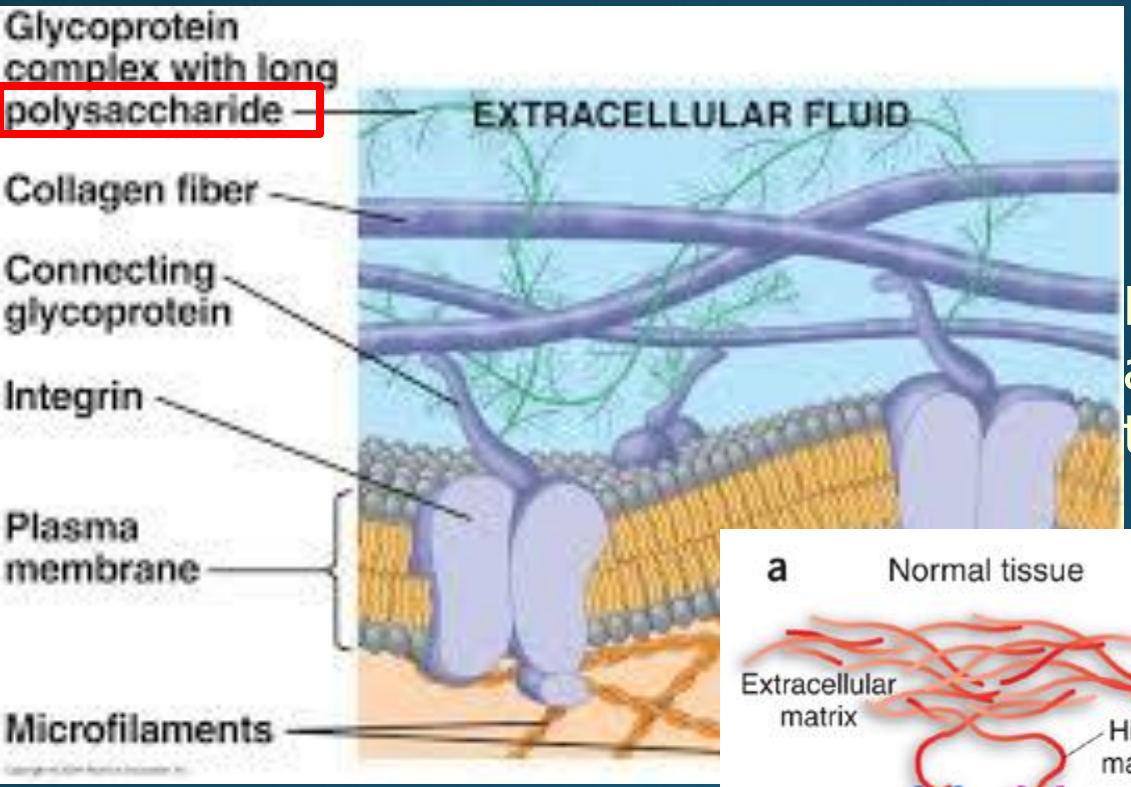
Energy Gap and Implications

- The continual accumulation of glucose metabolites will lead to cell death if the accumulation is not channeled out
- What is the connection between the accumulation of glucose metabolites and cell proliferation?
- Oncogenes have been proposed to be the link but that may not work since most of the affected cells are probably not signaled to proliferate, which involves many parts and coordination

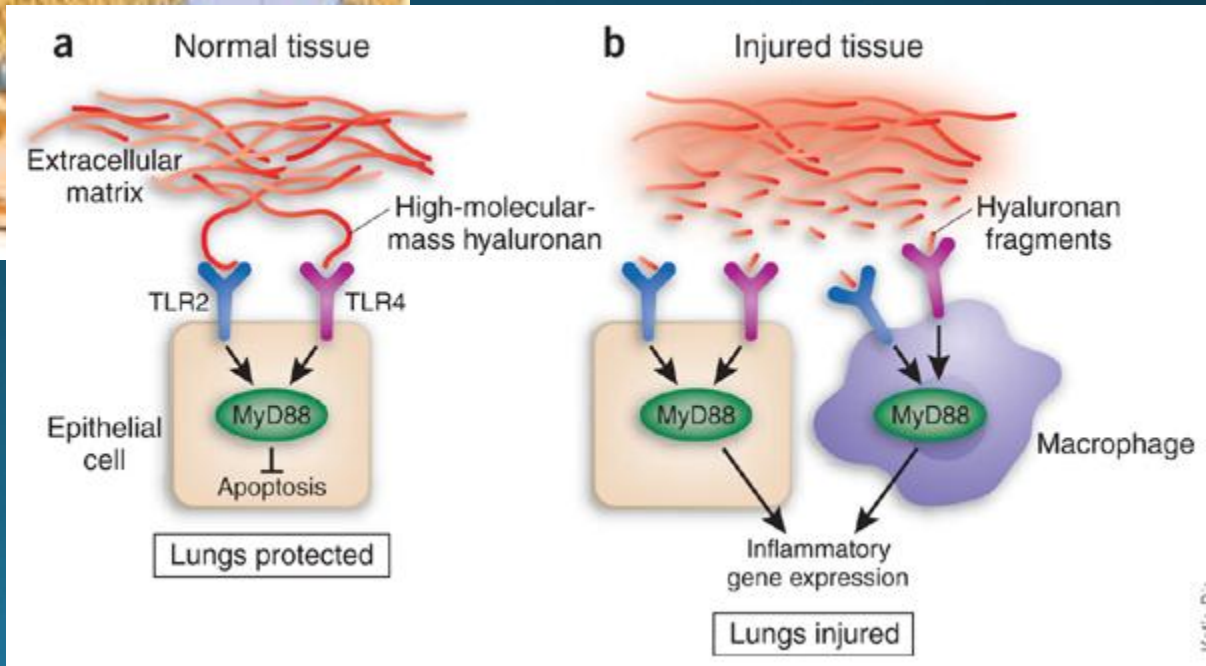
Accumulation and Cell Proliferation



Hyaluronic Acid and Function

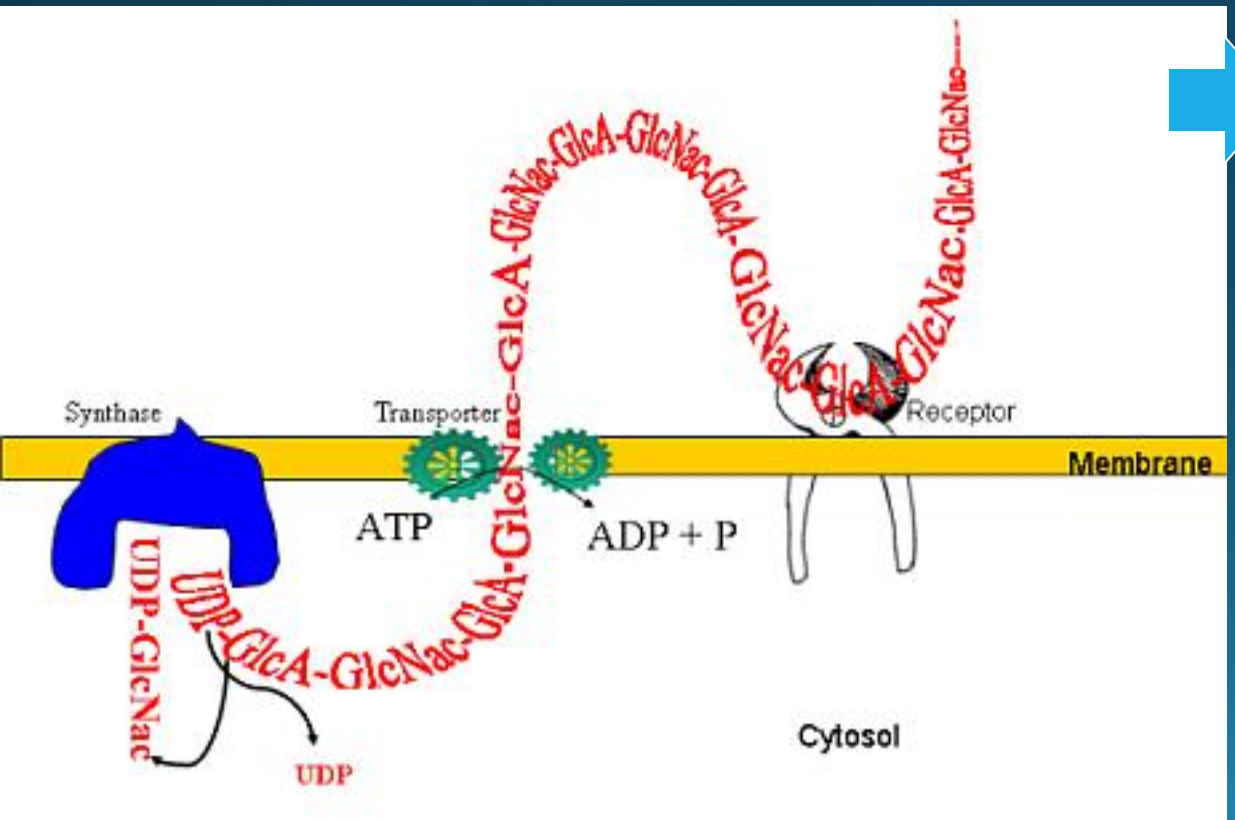


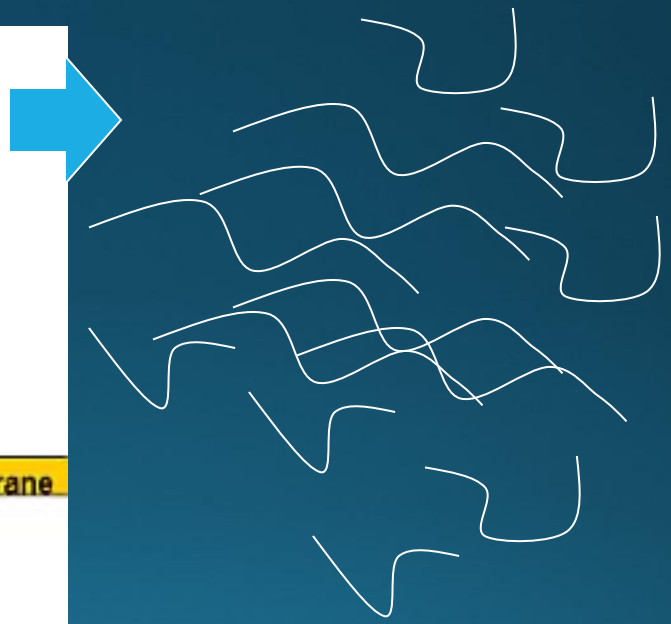
Hyaluronic acid fragments serve as a wide range of signals for tissue injury and repair



Hyaluronic Acid and Hypoxic Cells

- Synthesized hyaluronic acids will be exported out of cells and then degraded into fragments, hence providing all the signals for continuous growth





Hyaluronic Acids and Tissue Repair

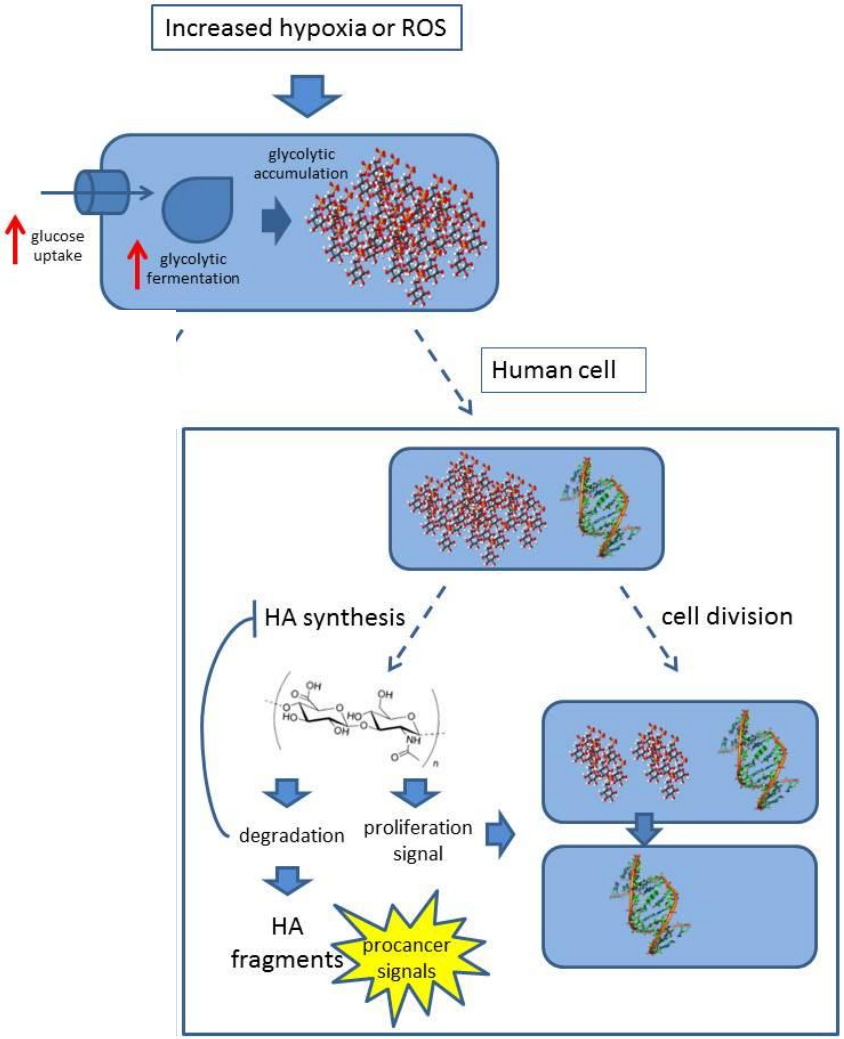
- Hyaluronic acids of different sizes serve as different signals for tissue injury and repair
  - Inflammatory signals to recruit immune cells
  - Cell survival through repressing apoptosis
  - Cell-cycle control
  - Cell growth
  - Loss of contact inhibition
  - Anchorage-independent growth
  - Angiogenesis
  - ….

Virtually all the signals needed for tumor cell proliferation

(Short) hyaluronic acids of different sizes serve as different signals, all related to tissue repair

Size (saccharides)	Function	References
High-molecular-mass HA > 1000–5000	Suppression of angiogenesis	Feinberg and Beebe (1983)
	Immune suppression	McBride and Bard (1979), Delmage et al. (1986)
	Inhibition of phagocytosis	Forrester and Balazs (1980)
	Suppression of HA synthesis	Lueke and Prehm (1999)
HA fragments		
~1000	Induction of inflammatory chemokines	Noble et al. (1993)
	Stimulation of PAI-1	Horton et al. (2000)
	Stimulation of urokinase	Horton et al. (2000)
10–40	Induction of CD44 cleavage	Sugahara et al. (2003)
	Promotion of tumor cell migration	Sugahara et al. (2003)
8–32	Stimulation of angiogenesis	West et al. (1985), Sattar et al. (1994), Slevin et al. (1998, 2002)
	Stimulation of tumor neovascularization	Rooney et al. (1995)
~15	Suppression of smooth muscle cell proliferation	Evanko et al. (1999)
12	Endothelial cell differentiation	Takahashi et al. (2005)
	Up-regulation of PTEN in tumor cells	Ghatak et al. (2002)
10	Displacement of matrix HA on oocyte surface	Camaioni et al. (1993)
	Displacement of proteoglycans from cell surface	Solursh et al. (1980)
6	Suppression of HA cable formation	de la Motte et al. (2003)
	Induction of NO and MMPs in chondrocytes	Knudson and Knudson (2004a, b)
	Induction of HAS2 in chondrocytes	Knudson and Knudson (2004a, b)
4–6	Induction of cytokine synthesis in dendritic cells	Termeer et al. (2000, 2002), Taylor et al. (2004)
	Transcription of MMPs	Fieber et al. (2004)
4	Up-regulation of Hsp 72 expression	Xu et al. (2002)
	Suppression of apoptosis	Xu et al. (2002)
	Induction of chemotaxis	R. Savani, personal communication
	Up-regulation of heat shock factor-1	Xu et al. (2002)
	Up-regulation of Fas expression	Fujii et al. (2001)
	Suppression of proteoglycan sulfation	Solursh et al. (1980)

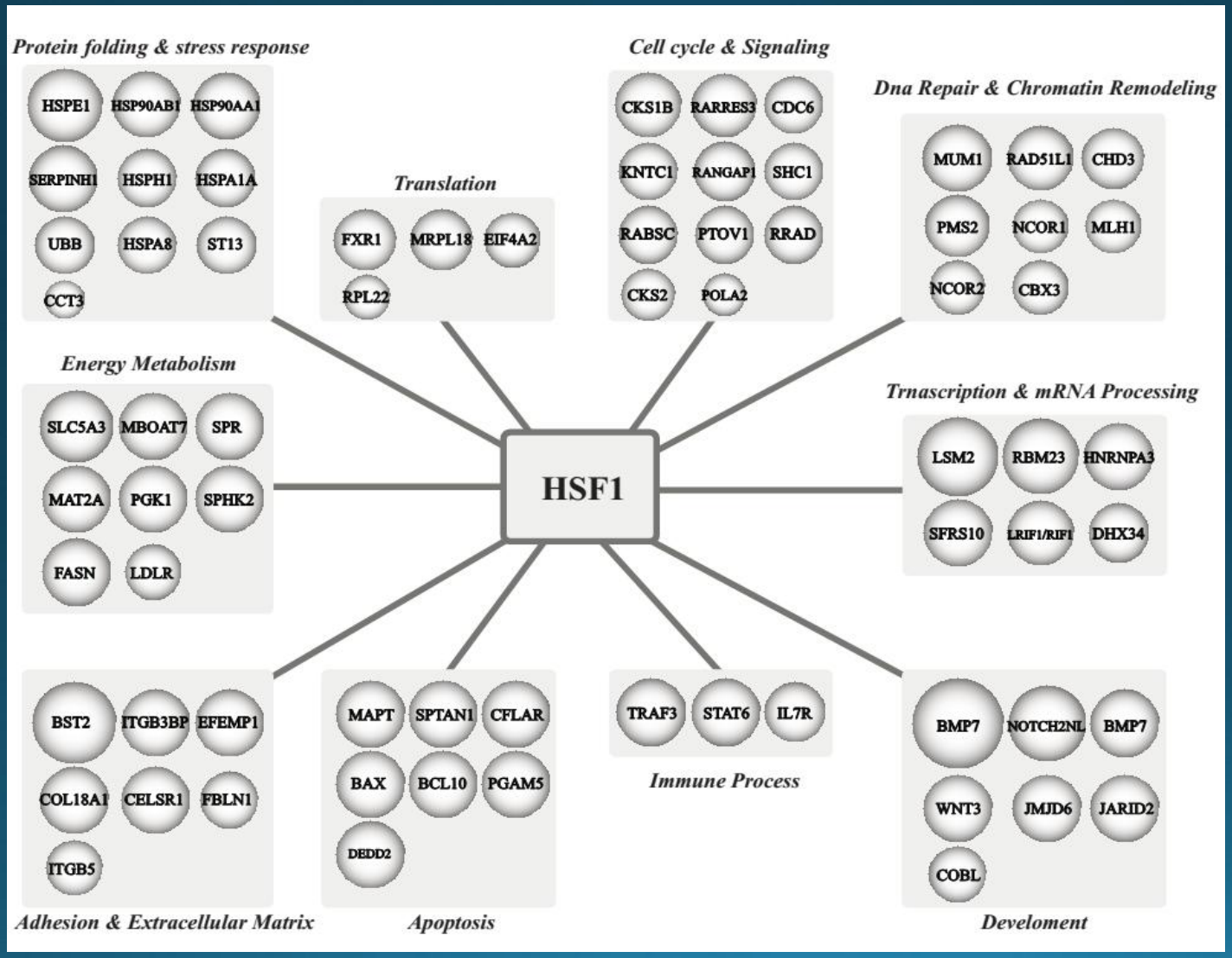
Hyaluronic Acids and Naked Mole Rat



cancer

Model: hypoxia -> accumulation of glycolytic metabolites -> hyaluronan synthesis and fragmentation -> signaling for tissue damage and repair; hence starts the tissue-repair process and opens the door for cell proliferation in a tissue environment

Master Regulator of Proliferation



Essential Roles of Hyaluronic Acids

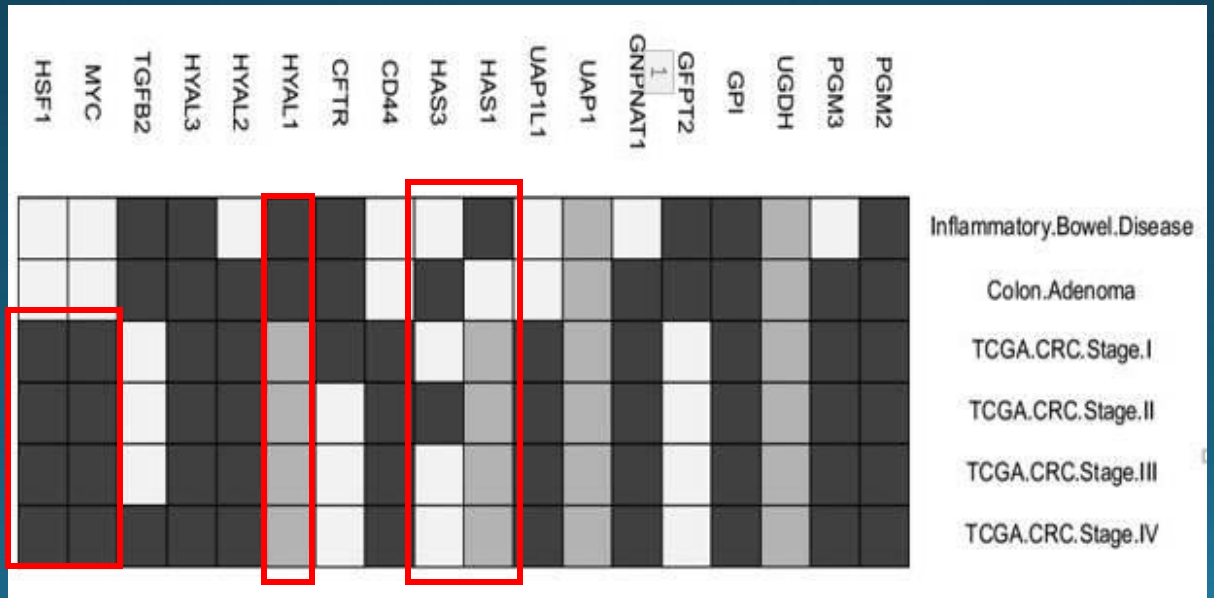
- Persistent production of hyaluronic acid production provides continuous signals for affected cells to
  - overcome virtually all tissue-level constraints for cell division such as contact-inhibition and anchor-dependence of ECM
  - overcome apoptosis
  - generate angiogenesis
  - .....
- Virtually all conditions for a cancer to take place

A good project to demonstrate this via a mouse cancer model

- Hypothesis: Persistent production of hyaluronic acid may be a prerequisite for large-scale genomic mutations since otherwise DNA repair mechanisms will fix all mutations

Synthesis of Hyaluronic Acid

- The key conditions for activation of hyaluronic acid synthesis pathway:
  - plenty of glucose metabolites, particularly G6P
  - hypoxic condition
  - availability of TGFβ (available when tissue is inflammatory)
- That is, under inflammation-induced persistent hypoxia, hyaluronic acid will be synthesized, exported and fragmented



Take-Home Message

- Omic data analysis and modeling have revealed considerable information about cancer development
- Considerable stresses are present in cancer-forming micro-environment, including hypoxia, oxidative stress and alkalization
- These stressors may have played key role in driving the development of cancer
- General principles for cancer evolution need to be well articulated

Homework

- Reading : Metabolic Reprogramming in Cancer Is Induced to Increase Proton Production, Cancer Research, 2020.
- Reading: Cancer Is A Survival Process under Persistent Microenvironmental and Cellular Stresses, GPB, 2023
- Reading Acid-base Homeostasis and Implications to the Phenotypic Behaviors of Cancer, GPB, 2023