亦敵亦友：探索微生物的世界

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TOPICS

- Introduction
- Beneficial microorganisms: microbial utilization & biotechnology
- Pathogenic microorganisms
- The genes we share with yeast
- Genes to genomics, proteomics & biological networks

Introduction: microorganisms

微生物之發現與微生物學之發展

什麼是微生物 (microorganisms / microbes) ？

細胞結構：原核與真核細胞

微生物在分類學上之地位

Microbiology

mikros = 微小 small

bios = 生命 life

logos = 科學

古代: 醬油、酒、麵包等釀造發酵食品

雷文霍克 (Antonie van Leeuwenhock):
十七世紀，使用自製的簡單顯微镜觀察
酵母菌、紅血球、原生動物以及細菌

自然發生說 (spontaneous generation):
認為生命乃是自然發生的

巴斯德 (Louis Pasteur, 1822-1895):
(1) 推翻了自然發生說, 而提倡了生物發生說
(biogenesis) ，確認了「生命源自於生命的觀念」

(2) 確認了酒精發酵與酵母的關係、殺菌問題等，
並說明了抗體（antibodies）在寄主內產生

http://en.wikipedia.org/

Pasteur Institute Study of biology, micro-organisms, diseases & vaccines

http://en.wikipedia.org/wiki/Pasteur_Institute

What are microorganisms / microbes ?

- 肉眼所無法見到之生物體

- 五大類微生物:

細菌 (Bacteria)　　原生生物 (Protoza)

藻類 (Algae)　　病毒（Virus）

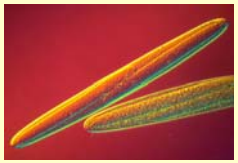
真菌 (Fungi): 酵母菌 (Yeast) & 黴菌 (Molds)

- "How big is a…. ?"
http://www.cellsalive.com/howbig.htm

Some "monster" bacteria:

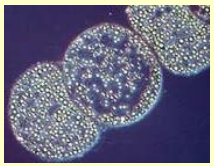
Epulopisium fishelson (1991): 刺尾魚(surgeonfish)之內臟 ，
紅海 / 澳大利亞, ~ 600 micrometers


Acanthurus lineatus, the striped surgeonfish



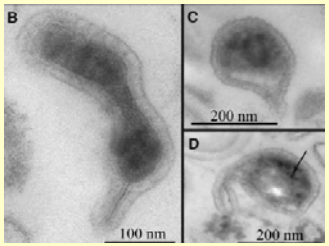
http://www.micro.cornell.edu/faculty/Angert/faculty.EAngert.html

Thiomargarita namibiensis (1999):
納米比亞之海岸, 100-750 micrometer



http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayfigure&book_id=4&fig_number=34&chap_number=2

How small an organism can be?



Three new archaea (by Dr. Brett Baker, University of California, Berkeley)
Acid water (pH = ~1.0)

Diameter: 200 nm (= 1/50,000 cm), ~ 1/5 of common bacteria
4,000,000 of them = 1 period (句點)

http://berkeley.edu/news/media/releases/2006/12/21_microbes.shtml

Cell structures: prokaryotic and eukaryotic cells

- 細胞: 生命之最基本單位
- 根據它們之細胞結構組成, 所有生活細胞可被分成兩類:

原核（Prokaryotic）& 真核（Eukaryotic）細胞

Prokaryotic cells	Eukaryotic cells
Bacteria	Animals Plants Fungi Protoza

Selected characteristics of prokaryotic & eukaryotic cells

	Prokaryote	Eukaryote
Cell size	Typically 0.2-2 μm in diameter	Typically 10-100 μm in diameter
Nuclear body	No nuclear membrane or nucleoli (nucleoid)	True nucleus consisting of nuclear membrane & nucleoli
DNA	Single molecule; not in chromosomes	Several chromosomes
Membrane-enclosed organelles	None	Mitochondria, Golgi complex, chloroplasts, vacuoles & others
Cell wall	Chemically complex; typically bacterila cell wall includes peptidoglycan	Thick or absent; chemically different

http://www.life.umd.edu/classroom/bsci424/BSCI223WebSiteFiles/ ProkaryoticvsEukaryotic.htm

微生物之分類: The three domain system

- Carl Woese & George Fox

- 此觀念是源自核醣體核醣核酸（ribosomal ribonucleic acid；rRNA）之研究
- 將生物分為三域 (domains):

Archaebacteria（太古生物）: methanogens, extreme halophiles, hyperthermophiles

Eubacteria（真細菌）: Gram (+) & Gram (-) bacteria

Eukaryotes（真核生物）: algae, protozoa, fungi, plants, animals

微生物之特性、細胞結構、營養需求與細胞生長

細菌之特性

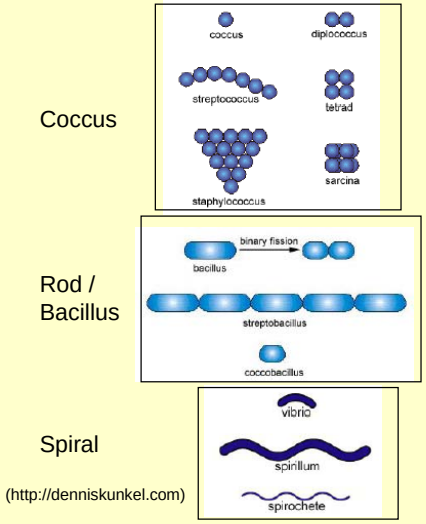
- 原核細胞
- 單細胞生物
- 一般比真核細胞較小
- 雖然很小、但是結構及生理能仍很複雜
- 大多數細菌具有下列其中一種形狀：

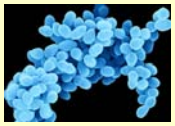
  球狀 （Coccus）
  桿狀 （Rod or bacillus）
  螺旋狀 （Spiral）

Coccus

Rod / Bacillus

Spiral

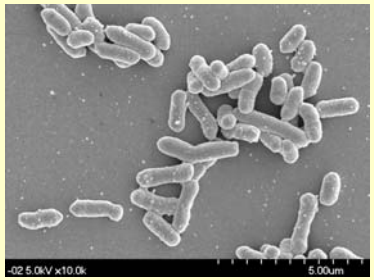




(http://denniskunkel.com)

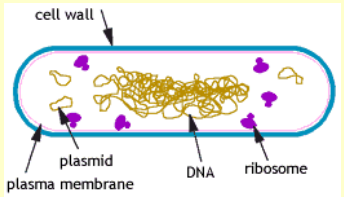
Scanning electron microscope
（掃描式電子顯微鏡）

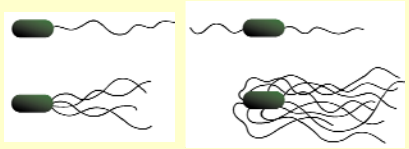
Escherichia coli



細菌之外觀結構

- 細胞壁（cell wall）、細胞膜（cell membrane）與外膜（outer membrane)
- 流體之細胞質（cytoplasm），其中含有一個 nuclear region (nucleoid 類核) & 核醣體（ribosomes）等
- 其他外在之結構（例如：鞭毛flagella）





http://bioweb.uwlax.edu/bio203/s2008/moder_just/adaptation.htm

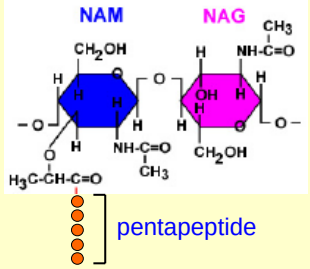
Transmission electron microscope
（穿透式電子顯微鏡）

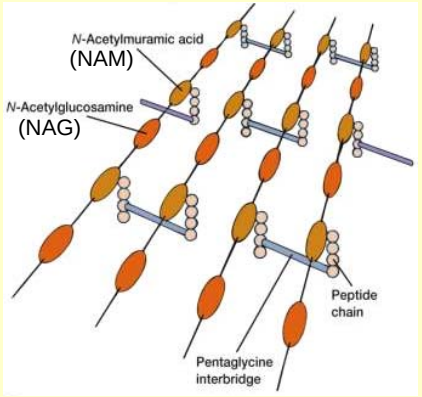
Pseudomonas aeruginosa

細菌之細胞壁 (cell wall)

- 由 **Peptidoglycan 肽聚糖 (或稱胞壁質murein) 多元體(polymer)** 所組成
- **Peptidoglycan多元體:** 由成鏈相同之peptidoglycan單元體組成
- **Peptidoglycan單元體 (monomer):**

N-acetyl glucosamine (NAG), N-acetyl muramic acid (NAM) and pentapeptide





Peptidoglycan (murein)

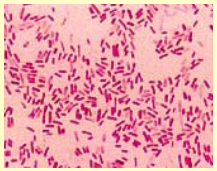
(Prescott *et al*., Microbiology, 2005)

格蘭陽性與格蘭陰性細菌

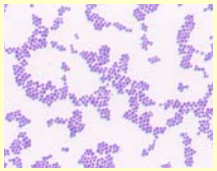
＜格蘭染色法＞
- Developed by Dr. Hans Christian Gram
- A valuable tool for clinical diagnostic & research
- Not all bacteria can be definitively classified by this method

- **Staining w/ crystal violet**(初染劑)
- **Treat with iodine**
- **Rinsed with alcohol**
- **Counterstain w/ safranine**(複染劑)

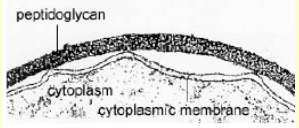
Gram (-) : Pink to Red



Gram (+): Dark purple

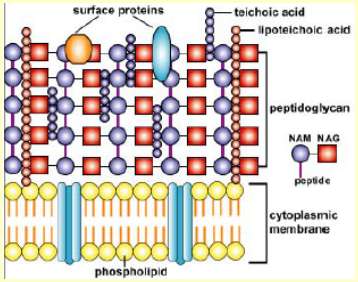


格蘭陽性細菌之細胞壁



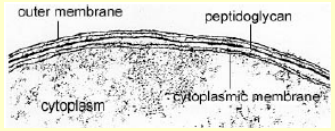
Peptidoglycan (60-80%)
Teichoic acids
Lipoteichoic acids
Proteins

- **Peptidoglycan 防止細胞因滲透壓造成溶菌（lysis)**
- **Techoic acids 可能幫助增強細胞壁之強度**
- **蛋白質之功能:**
  - 酵素
  - adhesins
  - invasins
  - 抗拒吞噬細胞之攻擊



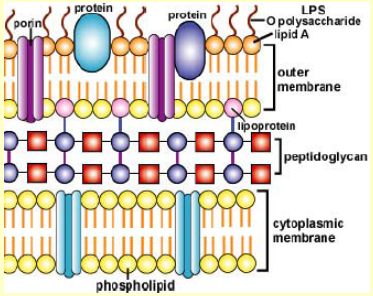
http://student.ccbcmd.edu/~gkaiser/goshp.html

格蘭陰性細菌之細胞壁



Peptidoglycan (10-20%)
Outer membrane (phospholipids, Lipoproteins, proteins & lipopolysaccharides, LPS)

- **Peptidoglycan 防止細胞因滲透壓造成溶菌（lysis)**
- **LPS是內毒素，可促進免疫之反應**
- **蛋白質之功能:**
  - 酵素
  - adhesins (附著因子)
  - invasins (侵入素)
  - 抗拒吞噬細胞之攻擊



http://student.ccbcmd.edu/~gkaiser/goshp.html

格蘭陰性細菌, 例如:

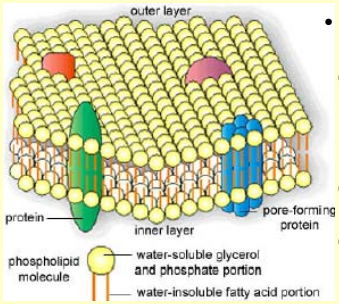
Escherichia coli（大腸桿菌）
Salmonella typhosa（傷寒菌）
Pseudomomas aeruginosa（綠膿桿菌）
Vibrio comma（霍亂菌）

格蘭陽性細菌, 例如:

Staphylococcus aureus（金黃葡萄球菌）
Bacillus subtilis（枯草桿菌）
Clostridium tetani（破傷風菌）

細菌之細胞膜 (cell membrane)

- 主要之化學組成份：磷脂質(phospholipid) & 蛋白質（proteins）



http://student.ccbcmd.edu/~gkaiser/goshp.html

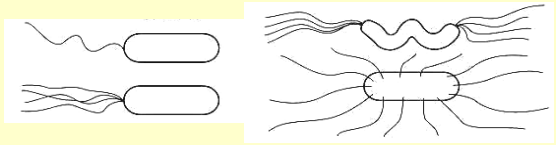
- 主要之功能：

(1)選擇性之通透膜：決定物質之進出（例如：營養素、氣體）

(2)廢物之排出、胞子之形成

(3)抗生素（例如：polymyxins）作用之目標

細菌之鞭毛 (flagella)



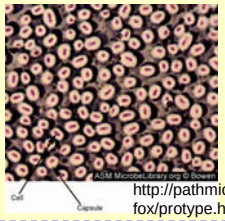
- 分為週鞭毛、端鞭毛、多鞭毛、單鞭毛等
- 細菌的運動器官，使細菌能在液體環境中泳動
- In general, counterclockwise rotation causes forward motion, clockwise rotation causing a tumble

E. coli flagella motility
http://www.youtube.com/watch?v=2P9hvlsF9_c

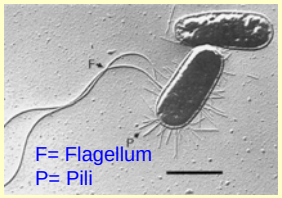
細菌之莢膜 (Capsule) & 纖毛 (Pili)

Capsule

- Resistance to phagocytosis
- Adherence to surfaces



http://pathmicro.med.sc.edu/fox/protype.htm



zbio.net/pictures/e_coli_pili.jpg

Pili

- Attachment to surface
- Bacterial mating

在細菌細胞質之內（within the cytoplasm)

- 細胞質：大多數細菌代謝反應發生之場所
- **Cytosol**：細胞質之液體成份
- **Nucleoid:** chromosome (染色體) 之所在，不具有核膜
- **Plasmids**（質體）: **small, closed circular DNA**
- **Ribosomes**（核醣體）：蛋白質之合成

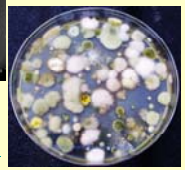
真菌之特性

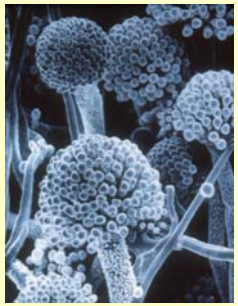
- 包含酵母菌（Yeast）與黴菌（Molds）
- 真核細胞: 有核膜及核仁，二或二條以上之染色體
- 有一個較堅固之細胞壁

Yeast

http://www.shieldmybasement.com/images/molddish1.jpg

Molds

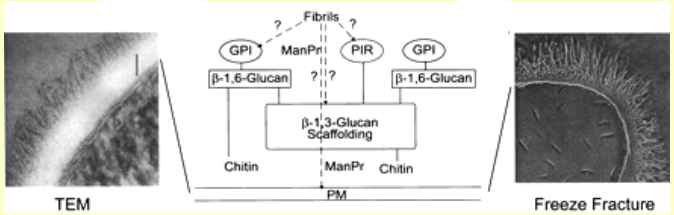




http://www.schoolproject2.ewebsite.com/photos/fungi/

真菌之細胞結構

- 細胞膜：phospholipid, proteins & glycoproteins
- 細胞壁： chitin and/or cellulose, proteins
  glucans (carbohydrate chains): glucose, mannose & N-acetyl-D-glucosamone



部份之功能:防止因滲透壓造成溶菌（lysis), 與寄主之接觸, drug targets

Membrane-bound organelles:

細胞進行代謝、提供能量、傳遞化學物質之場所

The nucleus: nuclear membrane, multiple chromosomes within

The endoplasmic reticulum (內質網): protein & lipid synthesis, transport molecules within the cells, molecules storage

The Golgi complex (高基氏體): sort proteins & lipids received from the ER, sort/package molecules into vesicle for transport to other part of the cell or secretion

Mitochondria (粒線體) and others

微生物之營養需求與細胞生長

微生物營養及環境之需求

- 對物理因素之需求

  Temperature　　Oxygen　　pH　　Osmosis

- 對營養成份及環境之需求

  Energy source: phototrophs & chemotrophs

  Carbon source: autotrophs (CO_2) & heterotrophs (organic comp'd)

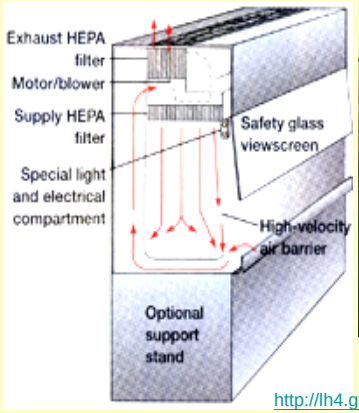
  Nitrogen source: nitrogen, nitrates, ammonia or organic N comp'd

  Minerals: S, P, K/Mg/Ca, Fe, trace elements

微生物培養基及其他器具之滅菌操作：高壓釜（autoclave）

121°C, 15 pounds/inch^2
15 分鐘

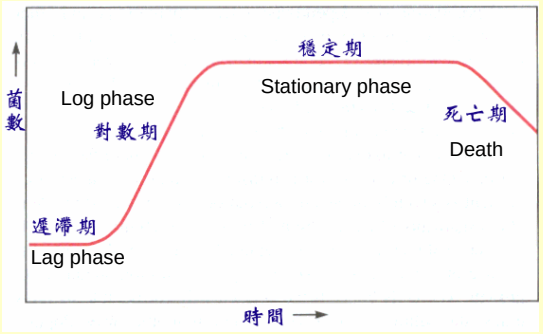
無菌操作台 (laminar flow), 去除 99.97 % > 0.3 μm 之物質



http://lh4.ggpht.com/_6vGOOBOkK4w/RbSezIDn-gI/AAAAAAAAFxE/GQ8j8HRd7hU/P1220005.JPG

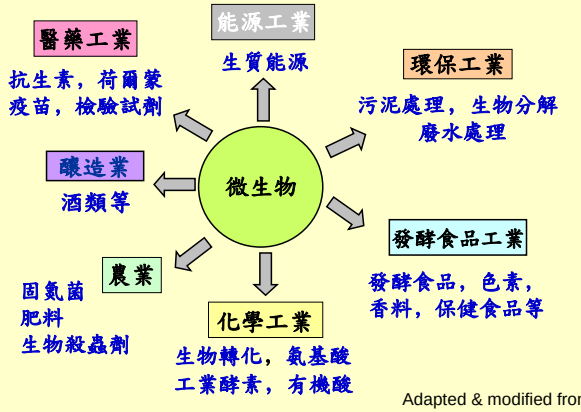
微生物之細胞生長

生長曲線 (growth curve):當一菌落被移到一新培養基時，菌落生長(菌數變化)之情形



微生物之利用與生物技術

微生物之利用



Adapted & modified from 劉英俊、汪金追、劉裕國 微生物應用工業

抗生素 (antibiotics) 之發現：penicillin 之故事

”由生物，尤其是微生物所生產之物質，阻止其他微生物與其他生活細胞機能、生長之物質”

- **Alexander Fleming (1929):** 發現在洋菜培養基上之葡萄球菌，在偶然進入之青黴菌(*Penicillium notatum*)菌落周圍,葡萄球菌呈透明狀。發現青黴菌可分泌抑制葡萄球菌生長之物質，即 **penicillin** 。

- **Florey & Chain (1940): 將 penicillin成功的純化與結晶化**

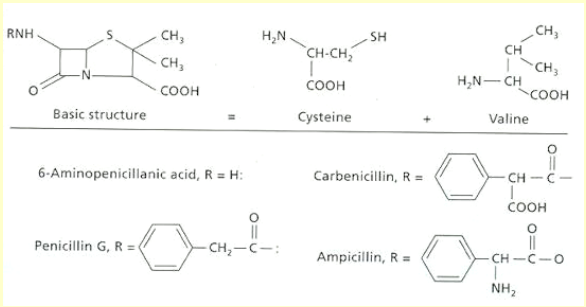
Penicillic 之故事（續）

- Penicillin prevents the cross-linking of small peptide chains in **peptidoglycan**
- Pre-existing cells are unaffected, but all newly produced cells grow abnormally, unable to maintain their wall rigidity, and they are susceptible to osmotic lysis.

Bacillus cereus

抗生素之發展與近況（current status)

- 抗生素之量產：1941年之後，研究大量生產之方法，促成抗生素工業之起始
- 抗生素之改良 (例：菌種及化學結構改良)與新抗生素之開發



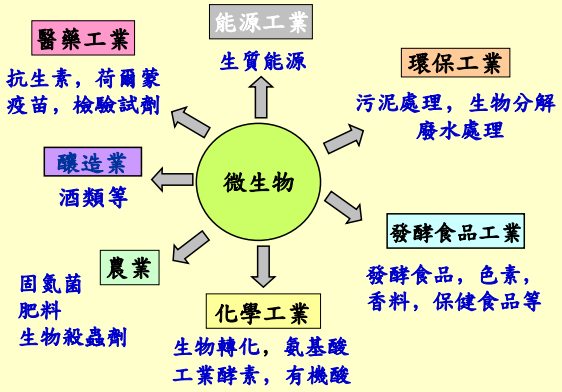
List of some antibiotics

http://en.wikipedia.org/wiki/List_of_antibiotics

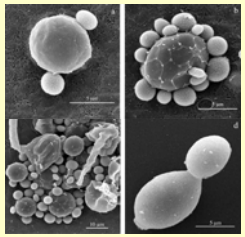
Antibiotics by class

Generic name	Brand names	Common uses[2]	Possible side effects[2]	Mechanism of action
Aminoglycosides				
Amikacin	Amikin	Infections caused by Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella* particularly *Pseudomonas aeruginosa*. Effective against Aerobic bacteria (not obligate/facultative anaerobes) and tularemia.	▪ Hearing loss ▪ Vertigo ▪ Kidney damage	Binding to the bacterial 30S ribosomal subunit (some work by binding to the 50S subunit), inhibiting the translocation of the peptidyl-tRNA from the A-site to the P-site and also causing misreading of mRNA, leaving the bacterium unable to synthesize proteins vital to its growth.
Gentamicin	Garamycin			
Kanamycin	Kantrex			
Neomycin	Mycifradin			
Netilmicin	Netromycin			
Streptomycin				
Tobramycin	Nebcin			
Paromomycin	Humatin			
Ansamycins				
Geldanamycin		Experimental, as antitumor antibiotics		
Herbimycin				
Carbacephem				
Loracarbef	Lorabid			prevents bacterial cell division by inhibiting cell wall synthesis

微生物之利用



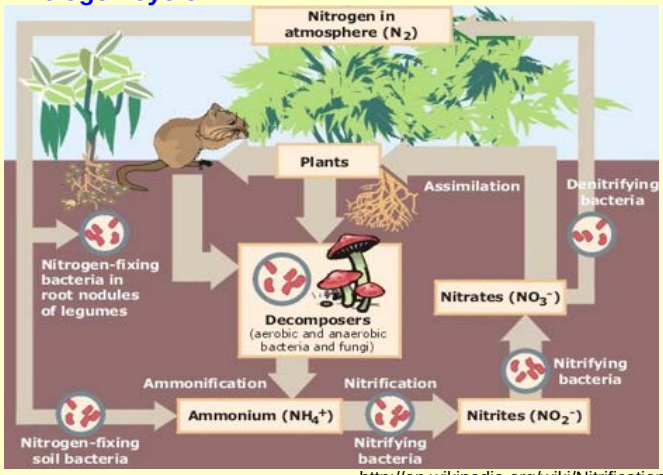
微生物之色素: 紅色酵母菌之故事



- 菌種: *Xanthophyllomyces dendrorhous*
- Astaxanthin-producing yeast
- Astaxanthin (蝦青素/蝦紅素):
  為脂溶性分子屬於類葫羅蔔素的一種
- Astaxanthin:
  (1) 對健康的可能益處 (2) 天然色素

http://www.microbelibrary.org/ASMOnly/details.asp?id=1971&Lang=

Nitrogen cycle



http://en.wikipedia.org/wiki/Nitrification

致病性微生物

Before birth: microorganisms-free

Normal microbiota / normal flora (正常微生物叢):
microorganisms that establish permanent colonies inside or on the body without causing disease

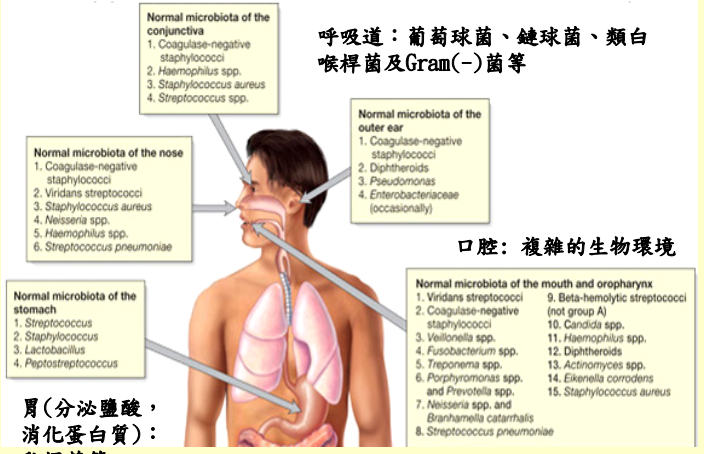
Skin and mucous membranes are readily colonized

Some benefits of the normal microbiota:

提供維生素等營養物供人類吸收與利用

幫助人類抵抗病原菌的侵襲及健全人類的免疫力

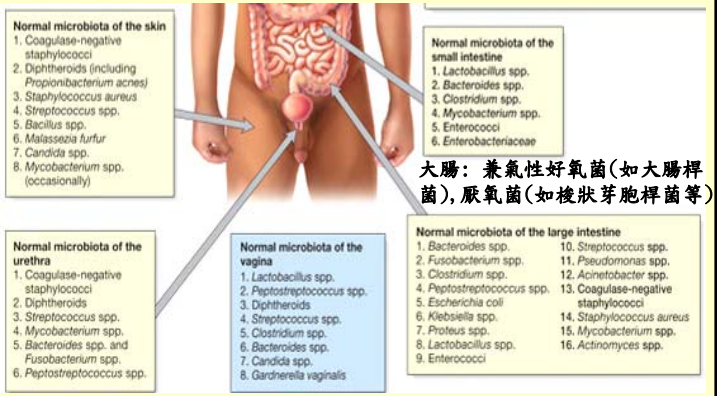
Normal microbiota of a human (1)



Prescott, Harley, and Klein's Microbiology (7th Ed.)

Normal microbiota of a human (2)

皮膚：包括幾種的葡萄球菌及厭氧性棒桿菌等



大腸：兼氣性好氧菌(如大腸桿菌),厭氧菌(如梭狀芽胞桿菌等)

生殖泌尿道：男性-厭氧性的Gram(-)桿菌和球菌;女性-厭氧性Gram(-)桿菌和球菌外，乳桿菌、酵母菌、鏈球菌和大腸桿菌等

Pathogens（致病菌）: disease-causing microorganisms

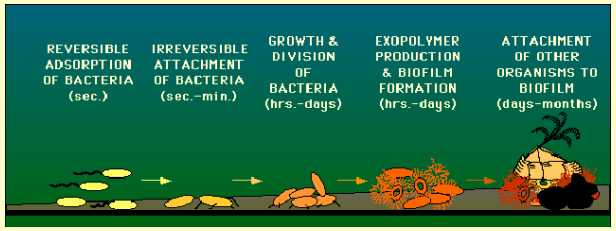
- 入侵人類身體或產生毒素
- 勝過免疫防衛系統並導致疾病

Opportunistic pathogens（伺機性病原菌）:

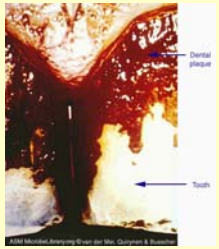
- 一些平時為normal microbiota 組成之微生物或是外來的微生物。
- 在宿主健康時，因normal microbiota的存在被控制在一定的繁殖數量與宿主共存，或被抑制生長而無法留存於宿主體內
- 當宿主的免疫力減弱或正常菌叢遭受破壞時，它們便能乘機大量繁殖並造成宿主生病。

http://microbiology.scu.edu.tw/lifescience/

Microbial biofilm（生物膜）



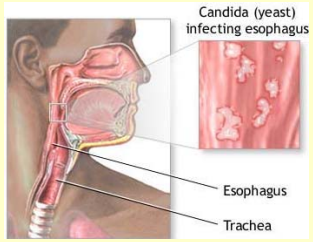
organized microbial communities encased in extracellular polymeric substance & associated with supporting surfaces (e.g. mucosal surface or medical devices)



Candida albicans: the major fungal pathogen of humans

- A member of normal microbiota & an opportunistic pathogen

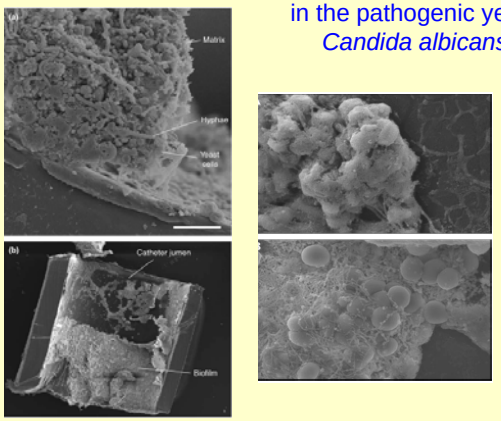
Pseudomembranous candidiasis (oral thrush)



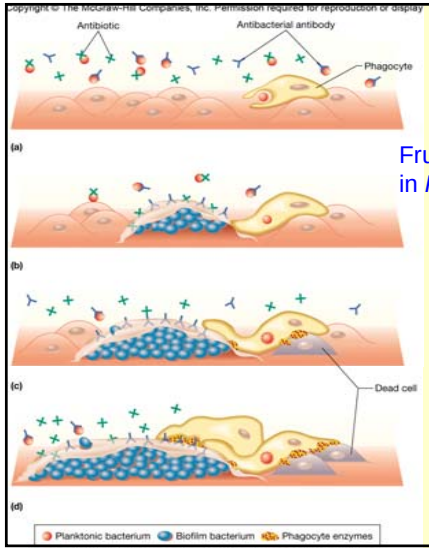
Candida Esophagitis

http://www.mdconsult.com

Biofilm formation in a medical catheter in the pathogenic yeast *Candida albicans*



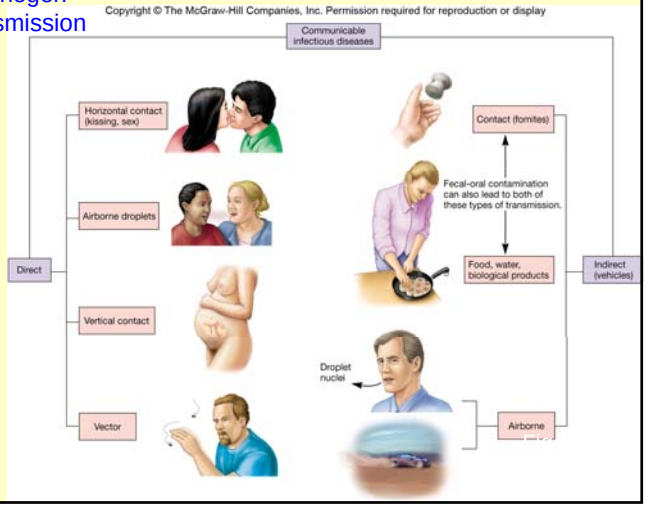
Nett & Andes. (2006) Curr Opion Microbiol, 9:340-345
Schinabeck *et al*. (2004) Antimicrob Agent Chemother, 48:1727



Frustrated phagocytosis in *Pseudomonas aeruginosa*

Prescott, Harley, and Klein's Microbiology (8th Ed.)

Pathogen transmission



Example: Airborne Transmission

- pathogen suspended in air and travels ≥1 meter

Prescott, Harley, and Klein's Microbiology (8th Ed.)

科克假說 (Koch postulates)

特異微生物引起特殊疾病時之
必備標準：

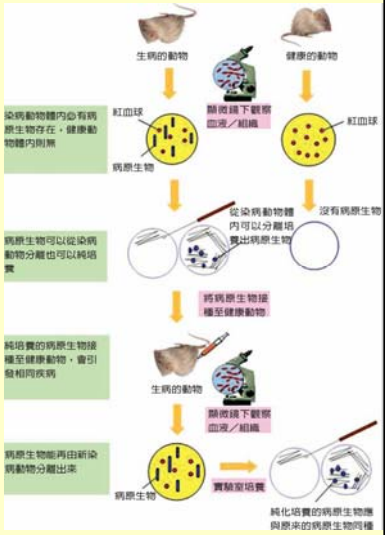
(1)某一特異微生物常與某一特定
疾病有關

(2) 此微生物可於染病動物中
分離出，且可以純培養

(3) 該微生物之純培養注入健康
動物時，將引起致病

(4) 自實驗感染之動物，可分離出
該注入之微生物

http://www.nsc.gov.tw/_newfiles/popular_science.asp?add_year=2006&popsc_aid=146



致病菌與疾病

疾病名稱	致病菌	一般感染人類方式
炭疽熱	*Bacillus anthracis*	直接接觸，孢子吸入或攝食
梨漿蟲病	*Babesia bovis, B.divergens, B. microti, B. equi*	傳染性壁蝨的咬傷
布魯士氏菌病(波形熱)	*Brucella melitensis, B. abortus, B. suis*	牛奶，直接或非直接接觸
彎曲桿菌病	*Campylobacter fetus, C. jejuni*	污染的水或食物
貓抓病	*Bartonella henselae*	貓或狗的抓傷
科羅拉多壁蝨熱	*Coltivirus*	壁蝨咬傷
牛痘病	Cowpox virus	皮膚擦傷
隱鞭孢子蟲症	*Cryptosporidium* spp.	與傳染性的小牛接觸
腦炎(加州型)	Arbovius	蚊子叮咬
腦炎(St. Louis)	Arbovius	蚊子叮咬
腦脊髓癌(Eastern equine)	Arbovius	蚊子叮咬
腦脊髓癌(Venezuelan equine)	Arbovius	蚊子叮咬
腦脊髓癌(Western equine)	Arbovius	蚊子叮咬
梨型鞭毛蟲病	*Giardia lamblia*	污水
鼻疽	*Pseudomonas mallei*	皮膚接觸，空氣吸入

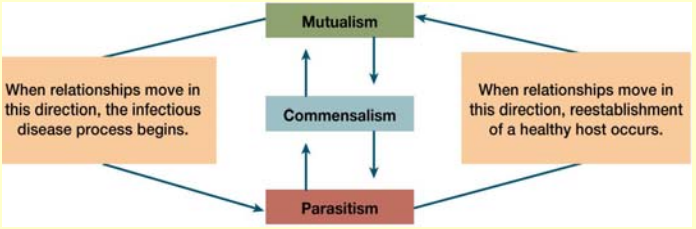
http://microbiology.scu.edu.tw/lifescience/

B型皰疹病毒性腦炎	*Herpesvirus simiae*	猴子咬傷，接觸來自猴子的物質
鉤端螺旋體病	*Leptospira interogens*	與污水、尿、感染的組織直接接觸
李斯特氏菌病	*Listera monocytogenes*	食物性感染
萊姆關節炎	*Borrelia burgdorferi*	感染的壁蝨咬傷
淋巴細胞性脈絡叢腦膜炎	Arobovirus	污塵的吸入、吃污染的食物
地中海熱	*Rickettsia conorii*	壁蝨咬傷
類鼻疽	*Pseudomonas pseudomallei*	節肢動物、水、食物
瘟疫	*Yersinia pestis*	跳蚤咬傷
鸚鵡熱	*Chlamydia psittaci*	直接接觸、呼吸道吸入
Q熱	*Coxiella burnetii*	吸入感染的泥土或塵埃
狂犬病	Rabies virus	患狂犬病動物的咬傷
回歸熱	*Barrelia* spp.	壁蝨或蝨的咬傷
立克次氏菌痘	*Rickettsia akari*	小鼠咬傷
落磯山脈斑點熱	*Rickettsia rickettsii*	壁蝨咬傷
沙門桿菌病	*Salmonella* spp.	直接接觸、食物
叢林型斑疹傷寒	*Rickettsia tsutsugamushi*	小鼠咬傷
結核病	*Mycobacterium bovis*	牛奶、直接接觸
兔熱病	*Francisella tularensis*	直接接觸感染的動物屍體、壁蝨咬傷、蒼蠅叮咬
斑疹傷寒熱	*Rickettsia mooseri*	蚤的咬傷
水泡性口腔炎	Virus（Rhabdovirus group）	直接接觸
黃熱病	Yellow fever virus	蚊子

(http://science.scu.edu.tw/micro/1024/micro_encyc/index.htm)

Symbiosis

（互利共生）



（寄生狀態）

微生物疾病之發生

影響因子: 環境因素、微生物致病力、寄主之抵抗力與敏感性

微生物之致病因子(virulence factors):

病原菌之附著力(adhension):莢膜、表面多醣物、附著性表面蛋白及菌絲等

穿透(entry):產生破壞組織或細胞的物質,
如:蛋白酶、脂酶等

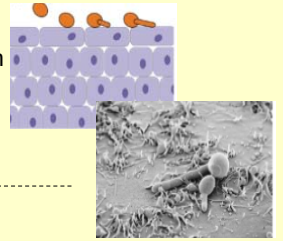
侵入(invasion)與增殖(multiplication)

毒素:外毒素—包括神經毒素、細胞毒素、腸毒素
內毒素—如Gram(-)細菌外膜之LPS

Contribution of virulence factors to *C. albicans* pathogenicity

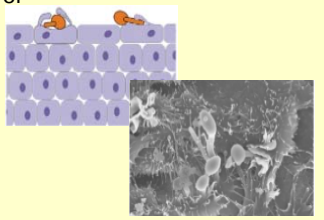
Stage 1: Colonization & attachment
Epithelial adhesion & nutrient acquisition

Virulence factors: Adhesins
Hydrolytic enzymes
Hyphae formation



Stage 2: Superficial infection
Epithelial penetration & degradation of host proteins

Virulence factors: Hydrolytic enzymes
Hyphae formation



Naglik et al. (2003) Microbiol Mol Biol Rev, 67:400
Zakikhany et al., (2006) Cell Microbiol, 9:2938

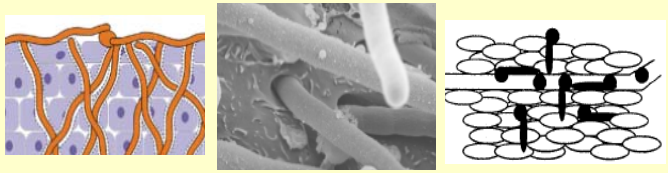
Stage 3: Deep-seated infection
Tissue penetration, vascular invasion & immune evasion or escape

Virulence factors: Hydrolytic enzymes Hyphae formation
Immunomodulation?

Naglik et al. (2003) Microbiol Mol Biol Rev, 67:400
Zakikhany et al., (2006) Cell Microbiol, 9:2938

Stage 4: Disseminated infection & tissue destruction
Endothelial adhesion, infection of other host tissues, activation of coagulation & blood clotting cascades

Virulence factors: Adhesins Hydrolytic enzymes
Hyphae formation
Immunomodulation?



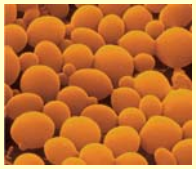
Naglik et al. (2003) Microbiol Mol Biol Rev, 67:400
Zakikhany et al., (2006) Cell Microbiol, 9:2938

微生物研究之前瞻

- 微生物在生物醫學上之應用：
  使用麵包酵母菌為模式生物去研究人類疾病

- 微生物基因體、蛋白質體與功能分析

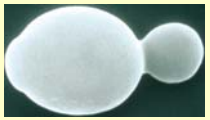
使用麵包酵母菌為模式生物去研究人類疾病



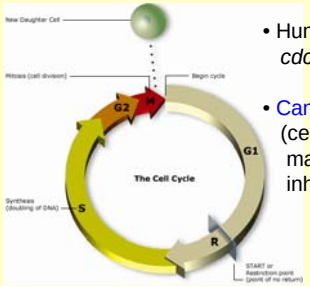
- 麵包酵母菌 (*Saccharomyces cerevisiae*):
  a one-cell (or unicellular) type of fungi
- They multiply as single cells that divide by budding 出芽 (as shown in the figure)

- Yeast as a model organism (模式生物):
  - it is easy & cheap to replicate
  - the average cell cycle for yeast is ~90 min (~24 h for human cells)
- Genome sequencing of *S. cerevisiae*
  - Just over 12 million base pairs, containing ~6,000 genes
  - About 20% of human disease genes have counterparts in yeast

Similarities yield clues to human disease: cancer as an example



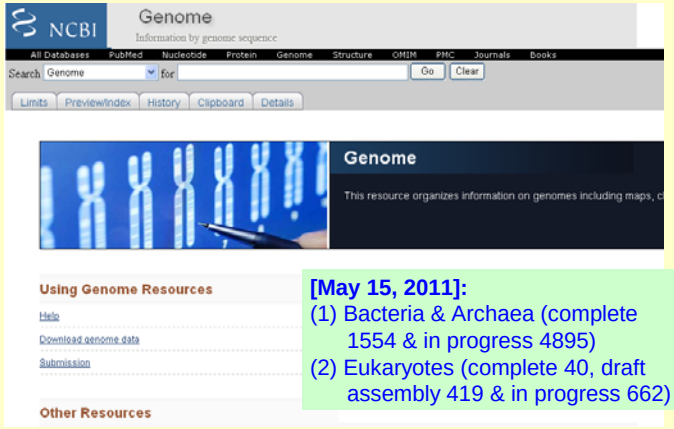
- As long as it has enough food, the yeast just eats & reproduces every ~ 90 min (cell-division cycle)
- Only abnormal yeast cells stop growing while their food supply is still plentiful (e.g. cell-division cycle or *cdc* mutants)



- Human equivalents of the yeast *cdc* genes are identified
- Cancer results from cell division gone wild (cell growth & multiply uncontrollably), may be due to genetic mutations that are inherited & caused by environmental factors

Current status of genome sequencing projects

http://www.ncbi.nlm.nih.gov/sites/entrez?db=genome

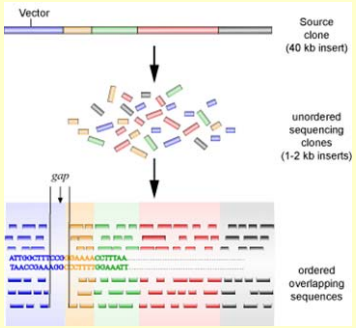


[May 15, 2011]:
(1) Bacteria & Archaea (complete 1554 & in progress 4895)
(2) Eukaryotes (complete 40, draft assembly 419 & in progress 662)

Overview of a microbial genome project

- DNA sequencing
- Genome assembly
- Gene annotation
- Databases

DNA sequencing: the shotgun method

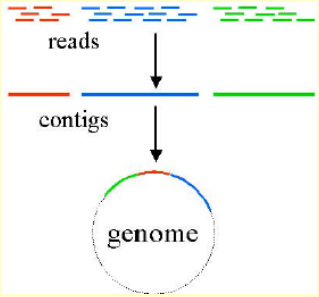


- Generate library representing whole genome
- Pick random clones for sequencing
- Assemble from overlap of clone sequences
- Rapid & simple

(http://www.bioteach.ubc.ca/Bioinformatics/GenomeProjects/)

Genome Assembly

- Sequencing reading from clones are overlapped – using sequence identities - to obtain large segments "Contigs"
- Resulting contigs are combined to assembly the whole genome
- By overlapping individual readings, a genome may be covered several times



(Bini, 2005)

Gene annotation

- Gene finding: determine putative open-reading-frames (ORFs)
- Translate ORFs
- Searching against databases of sequences

  homolog (hit a protein of known function)
  conserved hypothetical protein (hit a protein of unknown function)
  hypothetical proteins (if no match)

- Assignments for molecular functions

  Functional assignments (Pfam, etc)
  Structural assignments (NCBI, PDB)

DNA (gene) —transcription / 轉錄→ RNA —translation / 轉譯→ Protein

Applications of genome sequences

- Characterization of metabolic & regulatory pathways
- Identification of targets for antimicrobial agents
- Study of antibiotic resistance
- Identification of virulence factors for microbial pathogens
- Comparative genomics & evolution studies & human diseases
- Global regulation of gene expression

Some tools for global analysis of gene functions

- DNA microarrays (基因晶片)

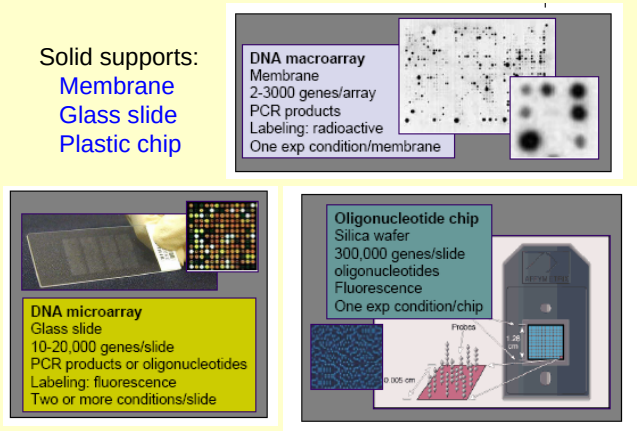
  Monitor the expression of thousands of gene all at once

- 2-D PAGE (二維式蛋白電泳分析) & mass spectrometry (質譜分析)

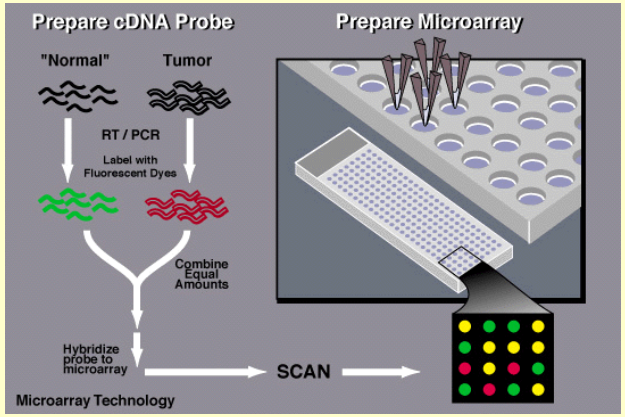
  Analysis of protein expression "Proteome" (蛋白組)
  ----→其分析研究即稱為蛋白組分析或蛋白質體(Proteomics)

DNA microarrays

Solid supports:
Membrane
Glass slide
Plastic chip



(Bini, 2005)



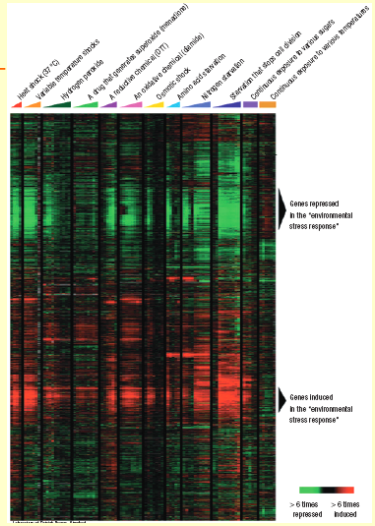
(http://www.accessexcellence.org/RC/VL/GG/microArray.html)

http://www.bio.davidson.edu/Courses/genomics/chip/chip.html

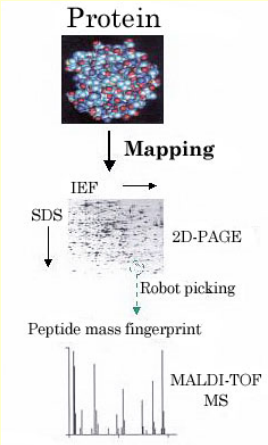
Clustering of gene expression

Comparing gene expression:

- Among different time points
- In response to different environmental conditions
- From different microbial strains (virulent strain *vs*. avirulent strain)
- Between normal cell *vs*. tumor cell
- Others



Proteome analysis

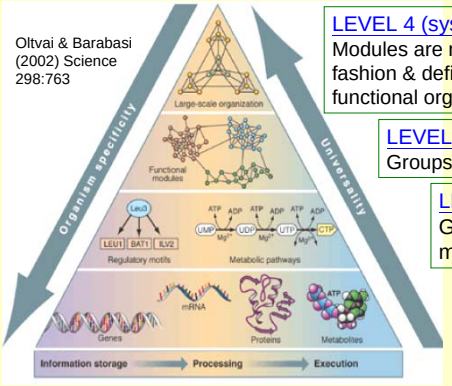


You can also compare proteome from different samples (e.g. virulent vs. avirulent strains)

Protein identification

Life's complexity pyramid: a system view of life

Oltvai & Barabasi (2002) Science 298:763



LEVEL 4 (systems)
Modules are nested in a hierarchical fashion & define cell's large-scale functional organization

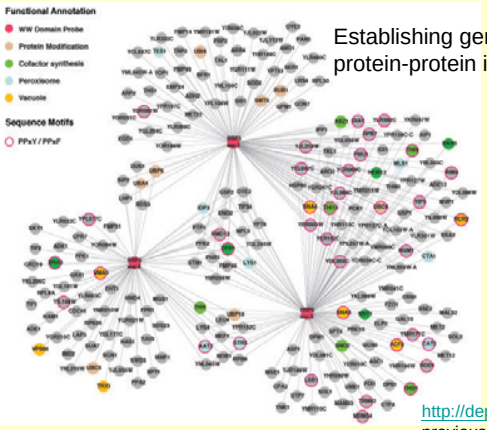
LEVEL 3 (functional modules)
Groups of nodes

LEVEL 2 (building blocks)
Genetic-regulatory motifs: metabolic pathways

LEVEL 1 (components)
Genome, transcriptome, proteome & metabolome

Individual components are likely unique to a given organism, the topologic properties of cellular networks sharing high similarity

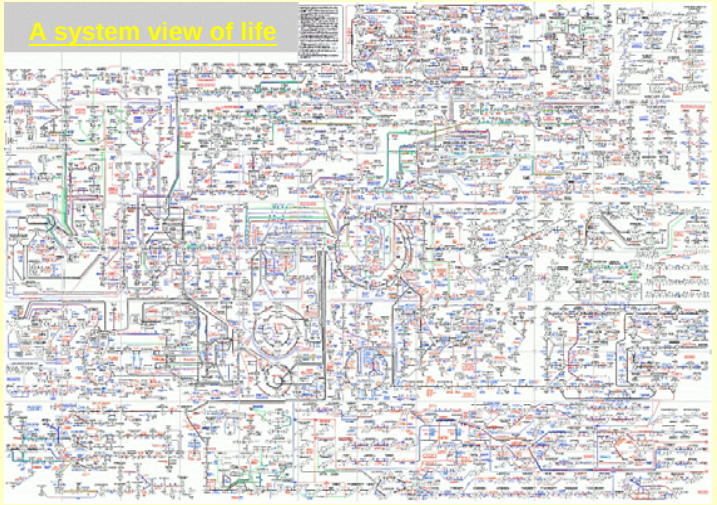
Biological networks & Systems Biology



Establishing gene regulatory & protein-protein interaction networks

http://depts.washington.edu/sfields/previous_projects/index.html

A system view of life



http://www.bio-pro.de/en/life/magazin/04653/index.html