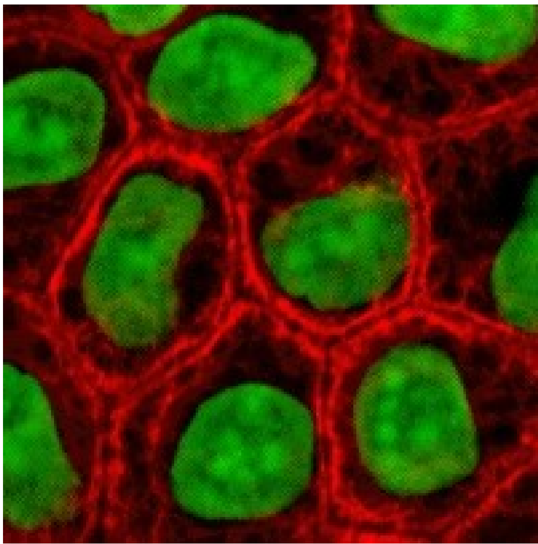
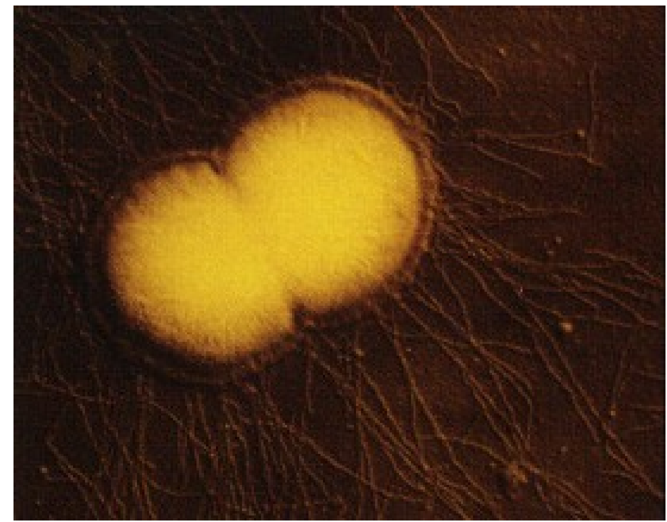
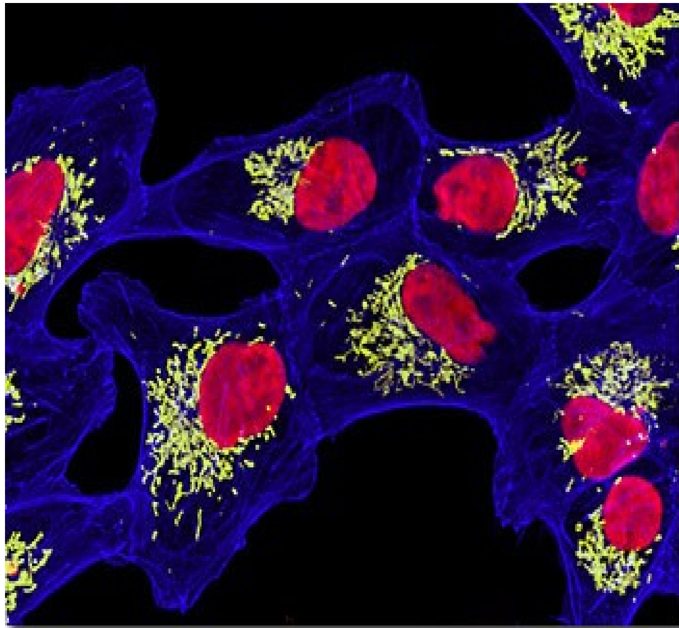
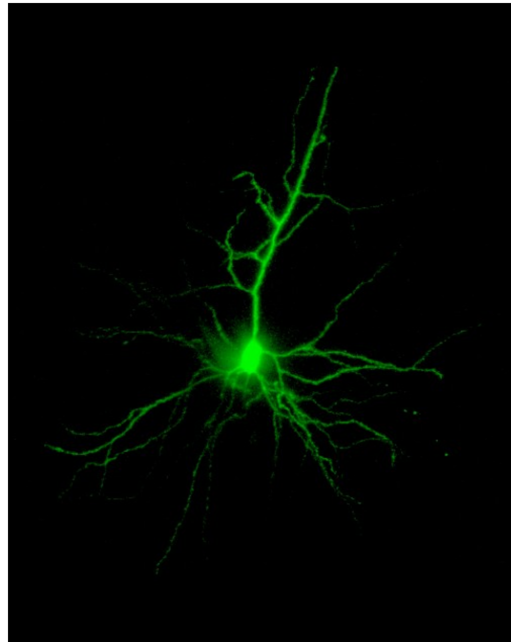
Modele elementare ale reactiilor enzimatice intracelulare

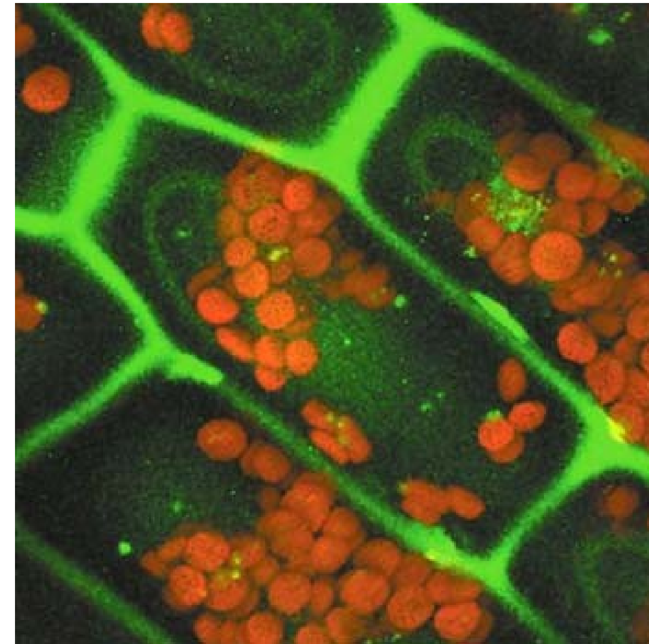




Celulele au forme foarte variate

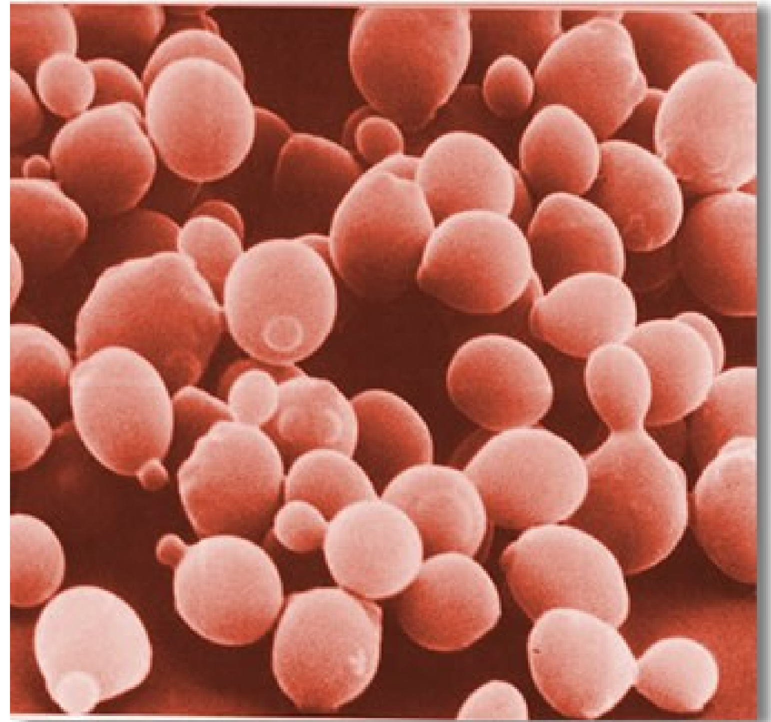






Imagini stereo a celulelor de *Saccharomyces cerevisiae*

file:///F:/CELL CHARACTERISTICS/YeastCellPicture_files/yeast_rg.jpg



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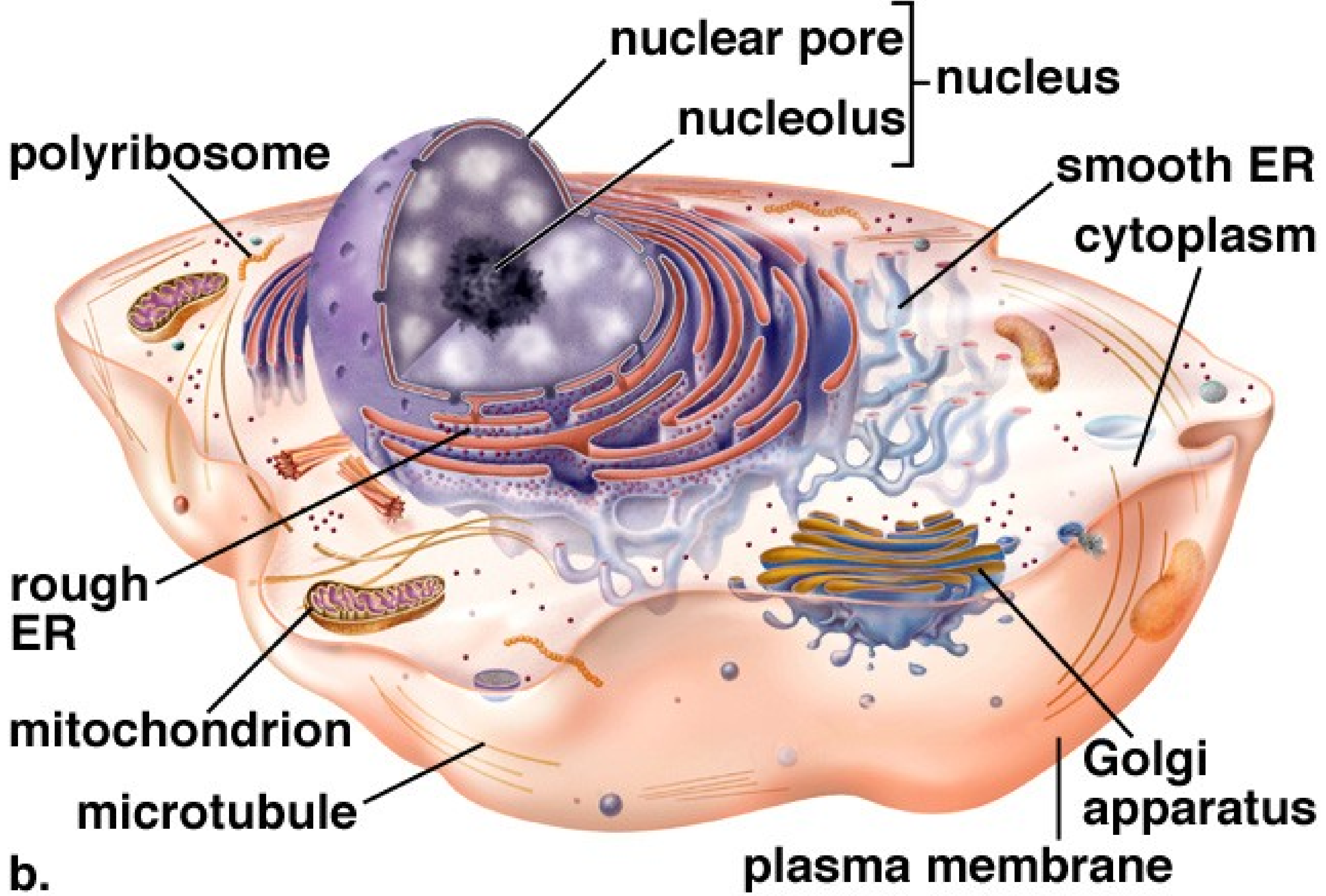
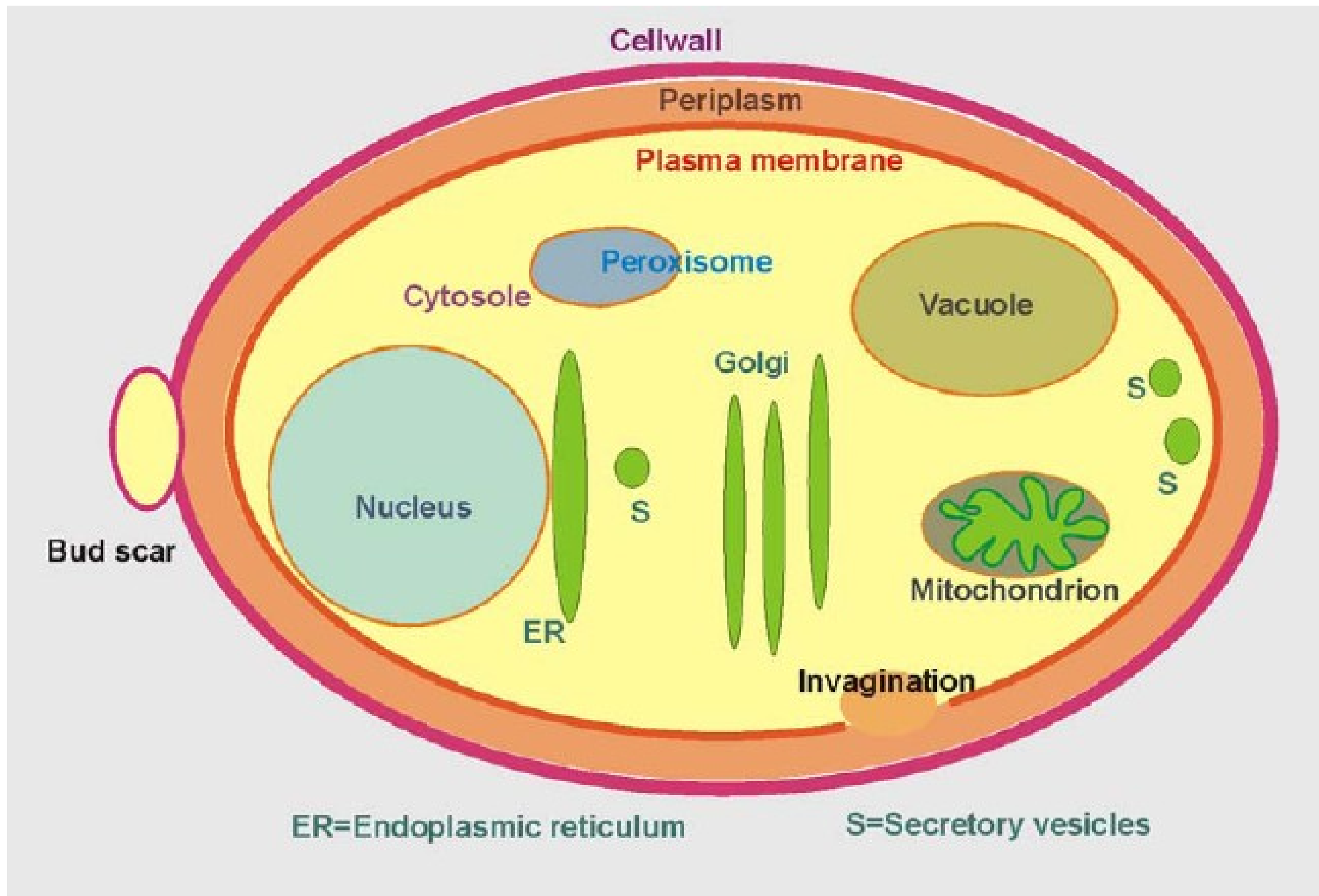
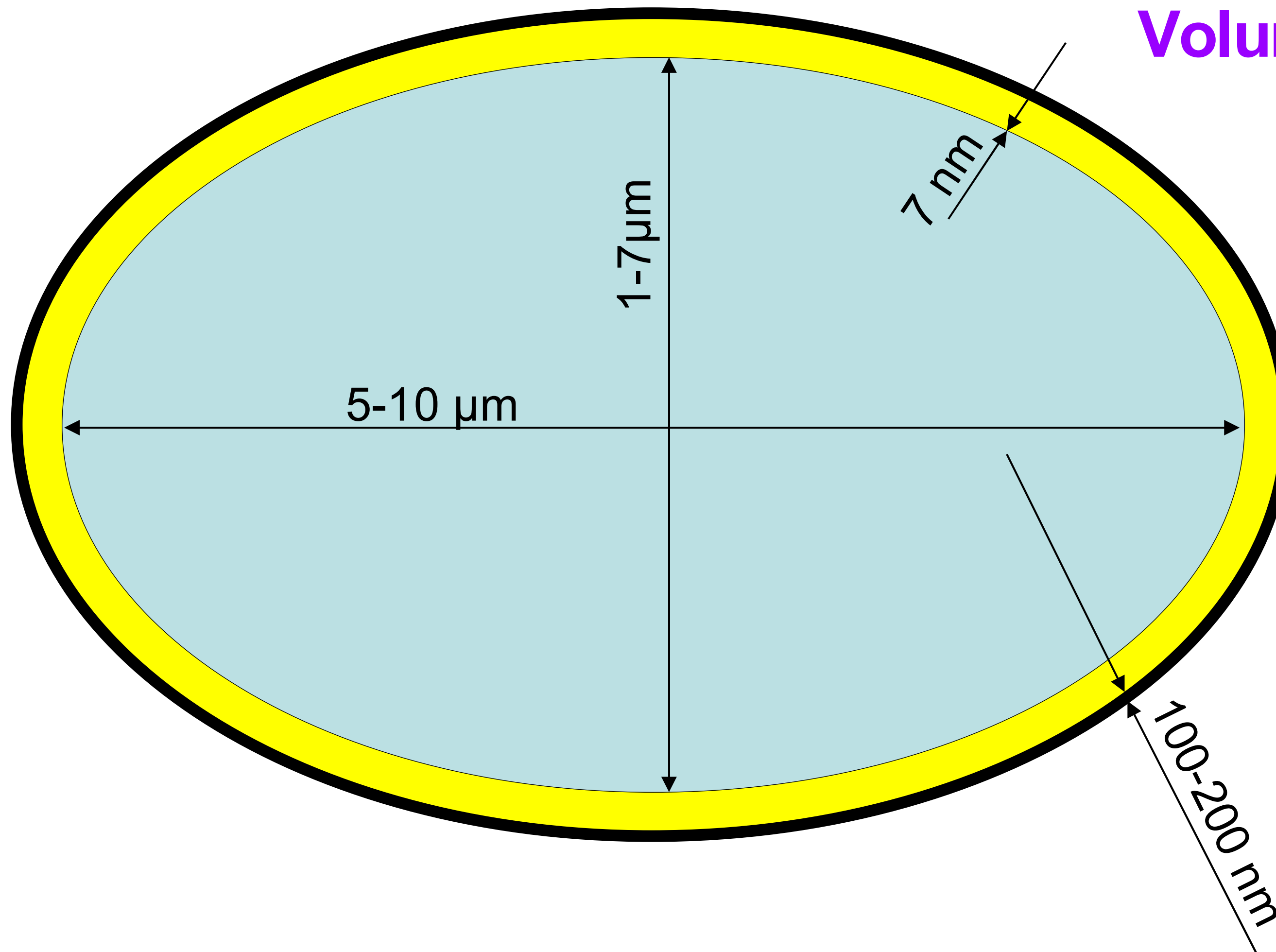
Animal cell



Reprezentarea schematica a celulei de drojdie



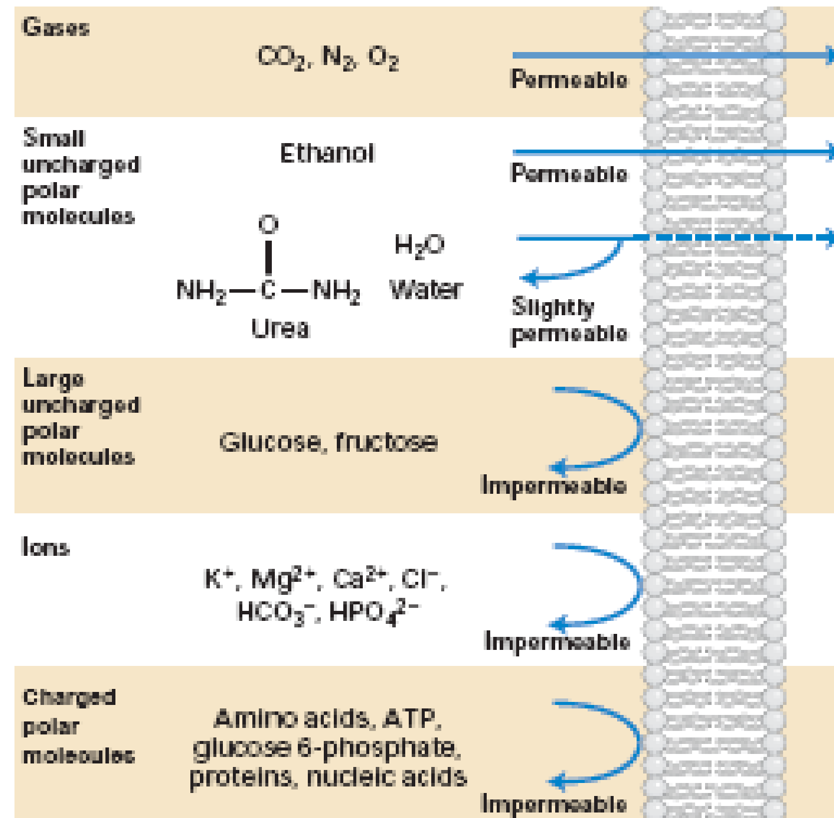
Parametri geometrici medii ai celulei de drojdie



Volum celular mediu:

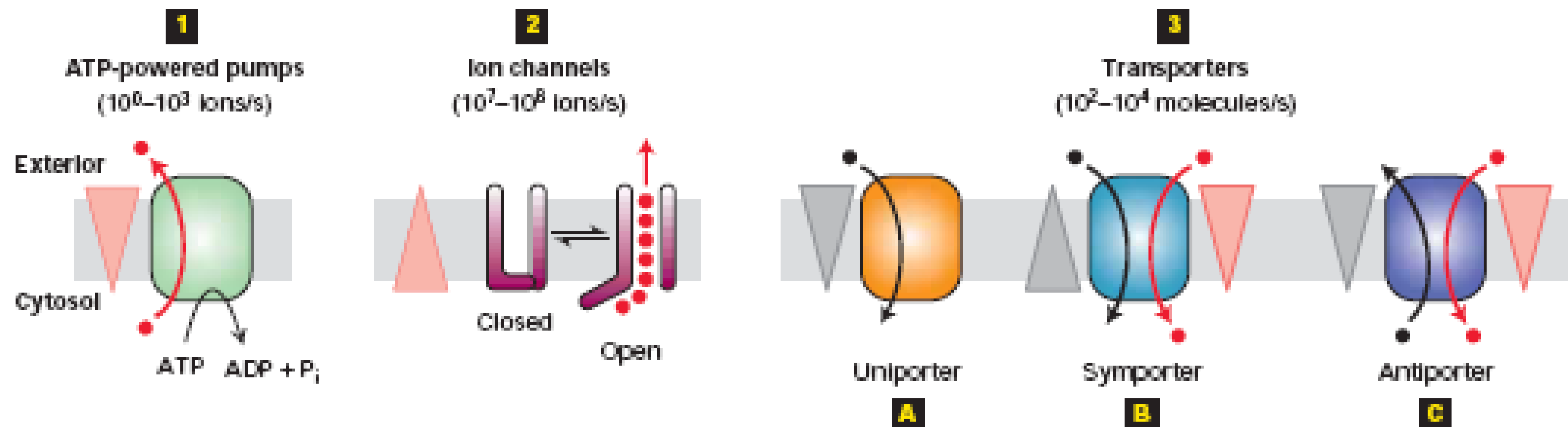
29-55 μm^3

Permeabilitatea membranei celulare la diferite molecule



▲ **FIGURE 7-1 Relative permeability of a pure phospholipid bilayer to various molecules.** A bilayer is permeable to small hydrophobic molecules and small uncharged polar molecules, slightly permeable to water and urea, and essentially impermeable to ions and to large polar molecules.

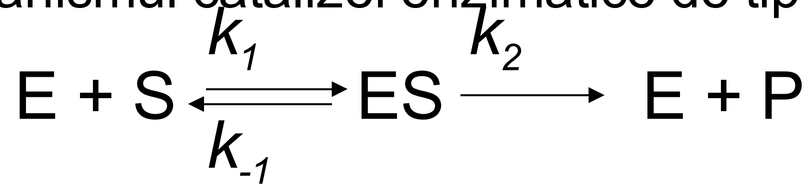
Clasificarea transportorilor celulari



▲ **FIGURE 7-2 Overview of membrane transport proteins.** Gradients are indicated by triangles with the tip pointing toward lower concentration, electrical potential, or both. **1** Pumps utilize the energy released by ATP hydrolysis to power movement of specific ions (red circles) or small molecules against their electrochemical gradient. **2** Channels permit movement of specific ions (or water) down their electrochemical gradient. Transporters, which fall into three groups, facilitate movement of specific small molecules or ions. Uniporters transport a single type of molecule down its concentration gradient **3A**. Cotransport proteins (symporters, **3B**, and antiporters, **3C**) catalyze the movement of one molecule *against* its concentration gradient (black circles), driven by movement of one or more ions down an electrochemical gradient (red circles). Differences in the mechanisms of transport by these three major classes of proteins account for their varying rates of solute movement.

Cinetica enzimatica de tip Michaelis-Menten

- Mecanismul catalizei enzimatice de tip Michaelis-Menten (M-M):



- Ipoteze folosite in deducerea ecuatiei Michaelis-Menten (sistem macroscopic):

i.) $[S]_0 \gg [E]_t$ ii.) $d[ES]/dt \approx 0$ iii.) validitatea legii actiunii maselor

$d[ES]/dt = k_1[E][S] - k_{-1}[ES] - k_2[ES]$ $[E] + [ES] = [E]_t$

$$\frac{d[P]}{dt} = v_0 = \frac{V_{max}[S]}{[S] + K_m}$$

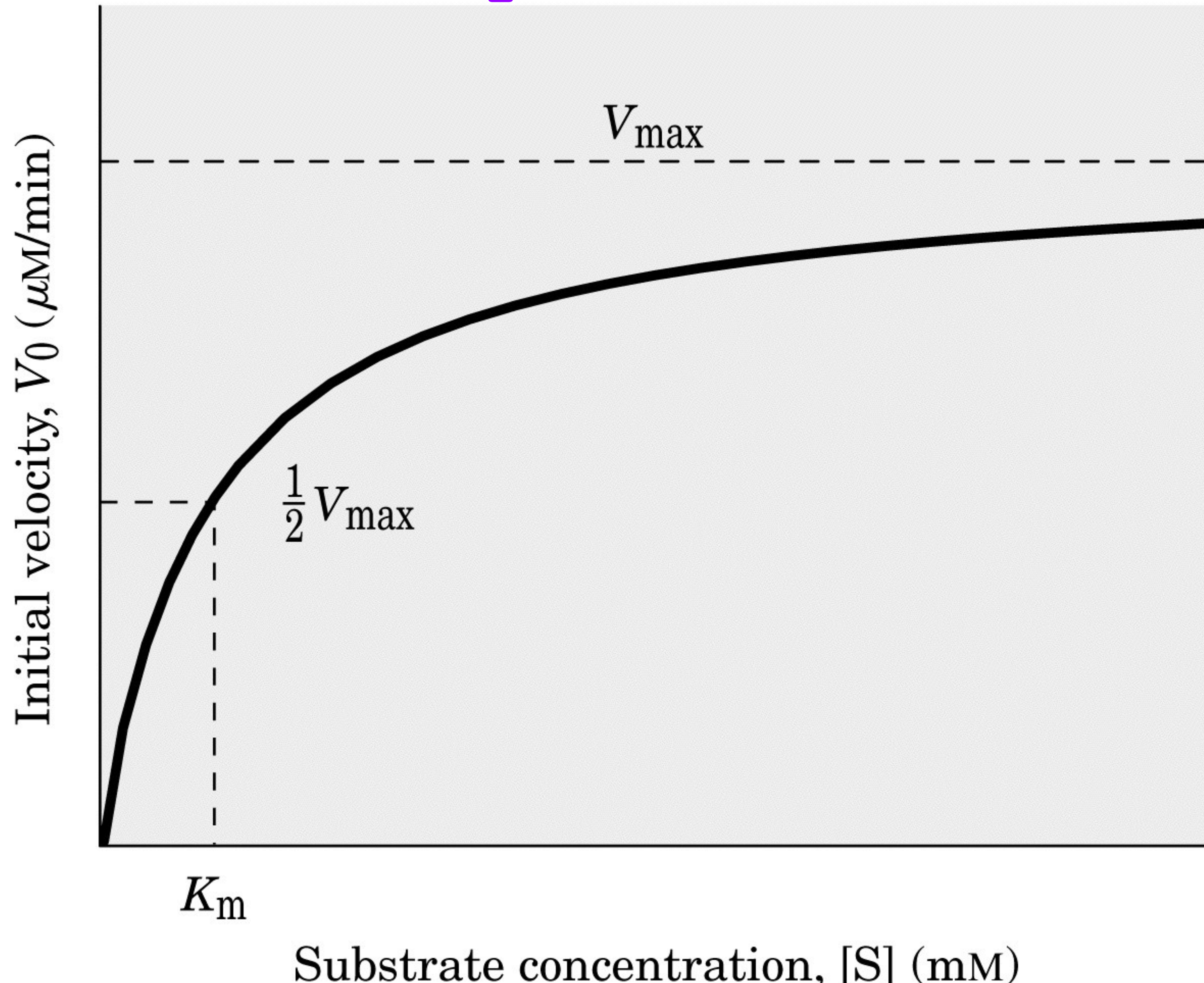
unde $V_{max} = k_2[E_t] = k_{cat}[E_t]$; $K_m = \frac{k_{-1} + k_2}{k_1}$

V_{max} este *viteza maxima*,

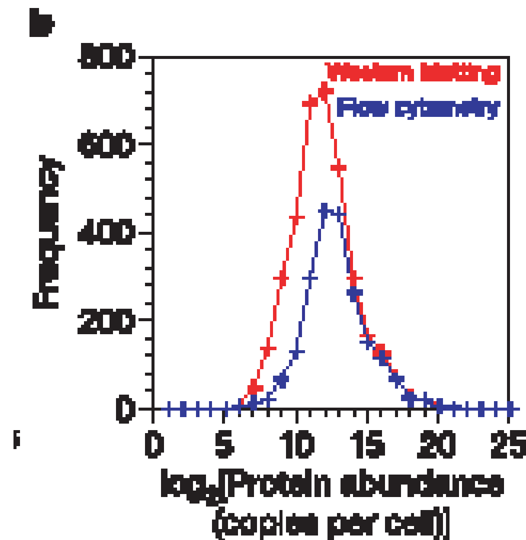
$k_2 = k_{cat}$ este *numarul de turnover* sau *constanta catalitica*,

K_m este *constanta Michaelis*

V_0= f([S]) la o reactie enzimatica de tip M-M este o curba hiperbolica de saturatie



Frecventa relativa a proteinelor celulare este variabila



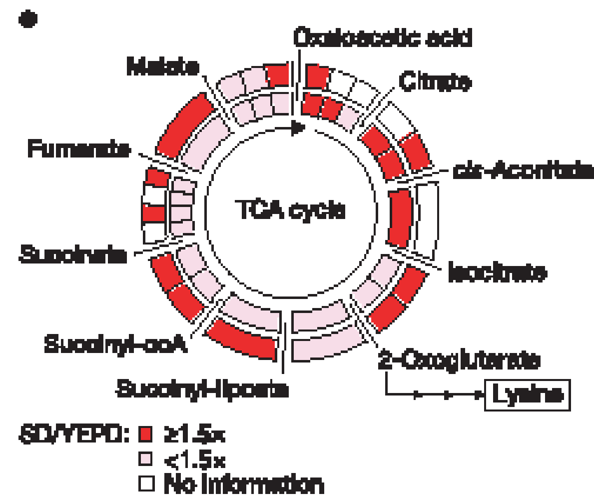


Figure 1 | Quantitative analyses of protein abundance using flow cytometry. **a,** Median fluorescence values for biological replicates of ~4,159 GFP-tagged strains grown in rich (YEPD) medium plotted against each other. M1, measurement 1; M2, measurement 2; a.u., arbitrary units. **b,** The frequency of detected, tagged strains is plotted as a function of the number of protein copies per cell ($\log_2$). TAP-tagged strains (red) were detected by western blotting[6]; GFP-tagged strains (blue) were detected by cytometry. The former approach detects essentially all tagged proteins present at more than 50 copies per cell, and thus provides a benchmark for evaluating the sensitivity of new approaches. **c,** Protein abundance measurements for 2,223 strains grown in rich (YEPD) and minimal (SD + Leu + Met + Ura) media. The standard errors of the measurements are also shown. Fluorescence from 313 strains can be quantified in YEPD but not SD, and 235 strains can be quantified in SD but not YEPD (data not shown). However, 2,763 strains (66% of the GFP library) can be quantified in at least one condition. **d,** The relationship between protein and mRNA ratios larger than two (colours other than grey) for cells grown in SD and YEPD. Changes in mRNA levels are largely captured by changes in protein levels (blue). In 21 cases, mRNA levels change without a corresponding change in protein levels. For 10 of these cases, protein levels do not change (orange), whereas for the remaining 11 cases (red), the proteins change in a direction opposite to that observed for the mRNA (see the main text). In contrast, changes in protein levels are not always captured by changes in mRNA levels, and we observed 131 instances of this behaviour (green). mRNA ratios were measured using DNA microarrays. Ratios were grouped operationally by lines having slopes of $+3/\frac{1}{3}$ (blue), $-3/-\frac{1}{3}$ (red), $+3/-3$ (green) and $\frac{1}{3}/-\frac{1}{3}$ (orange). **e,** Schematic showing that 11 out of 14 tricarboxylic acid (TCA) cycle proteins quantified by cytometry show greater than 1.5-fold induction in SD compared to YEPD (red segments, outer circle), but only 4 out of 19 mRNAs quantified by microarray analysis show induction at a similar level (red segments, inner circle). Note that Aco1 is used twice in the TCA cycle.

Zgomotul biologic al frecventei unei proteine depinde de functia ei biologica

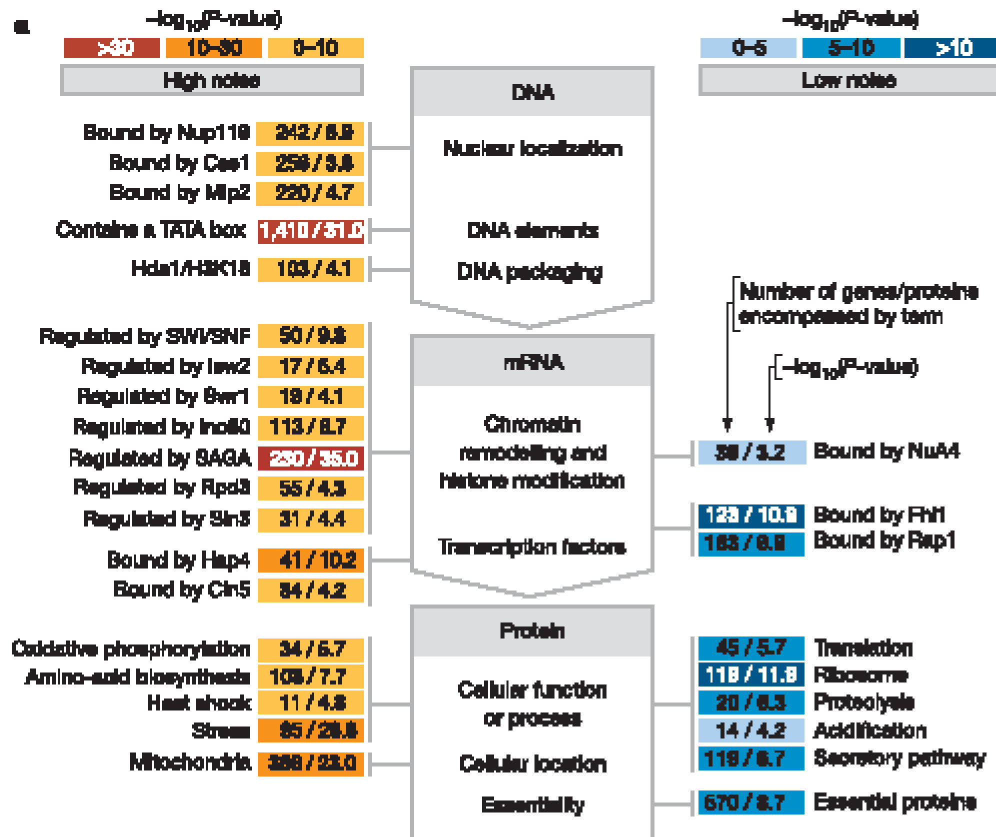
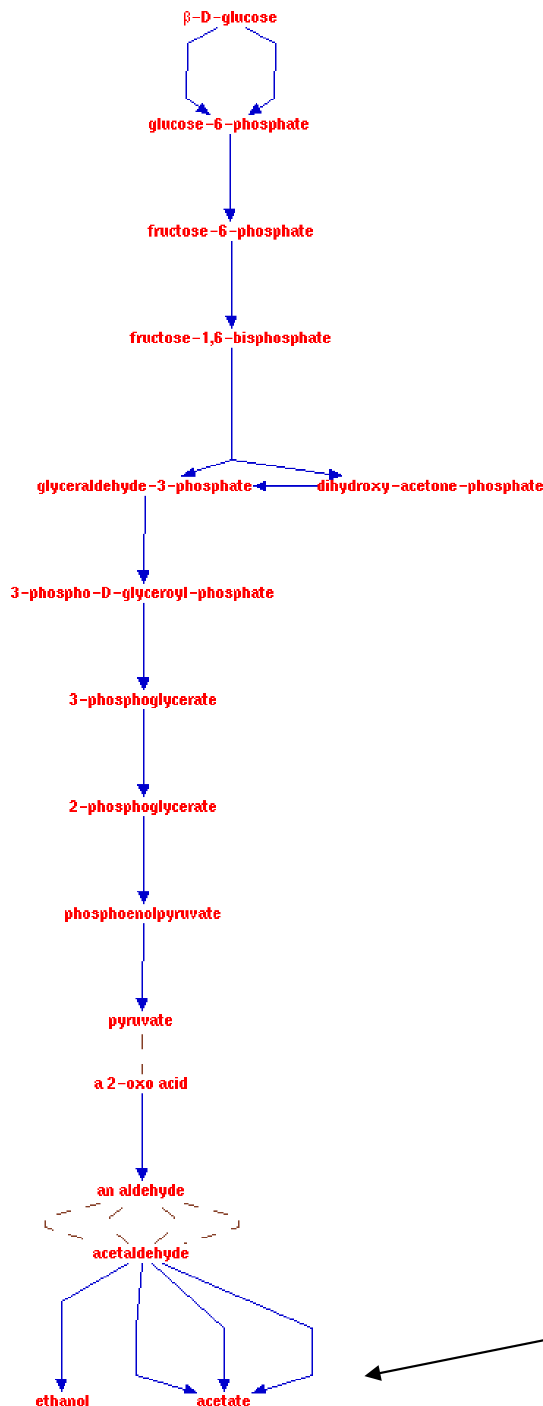


Figure 4 | Overview of major factors contributing to biological noise. **a**, Proteins targeted by chromatin remodelling complexes exhibit large variation whereas proteins participating in translation exhibit low variation (see also Supplementary Notes 2, Supplementary Fig. S14, Supplementary Table S5 and Supplementary Table S6). **b**, mRNA or protein copy number

Un exemplu de secventa de reactii enzimatice, cu o importanta deosebita pentru orice tip de celula vie

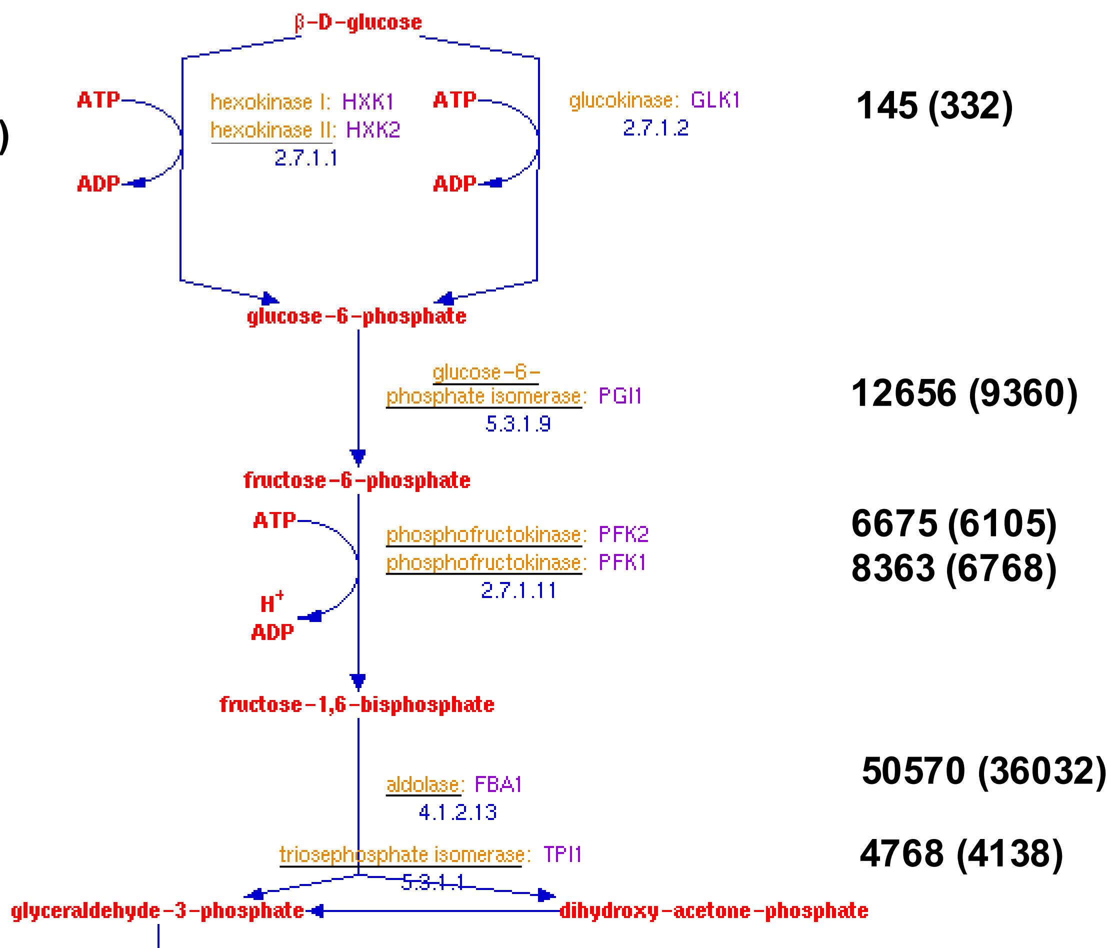


Glicoliza este calea metabolica de transformare a glucozei in piruvat, printr-o secventa de reactii catalizate enzimatic

In cazul particular al glicolizei la drojdie, piruvatul este convertit partial in alcool etilic

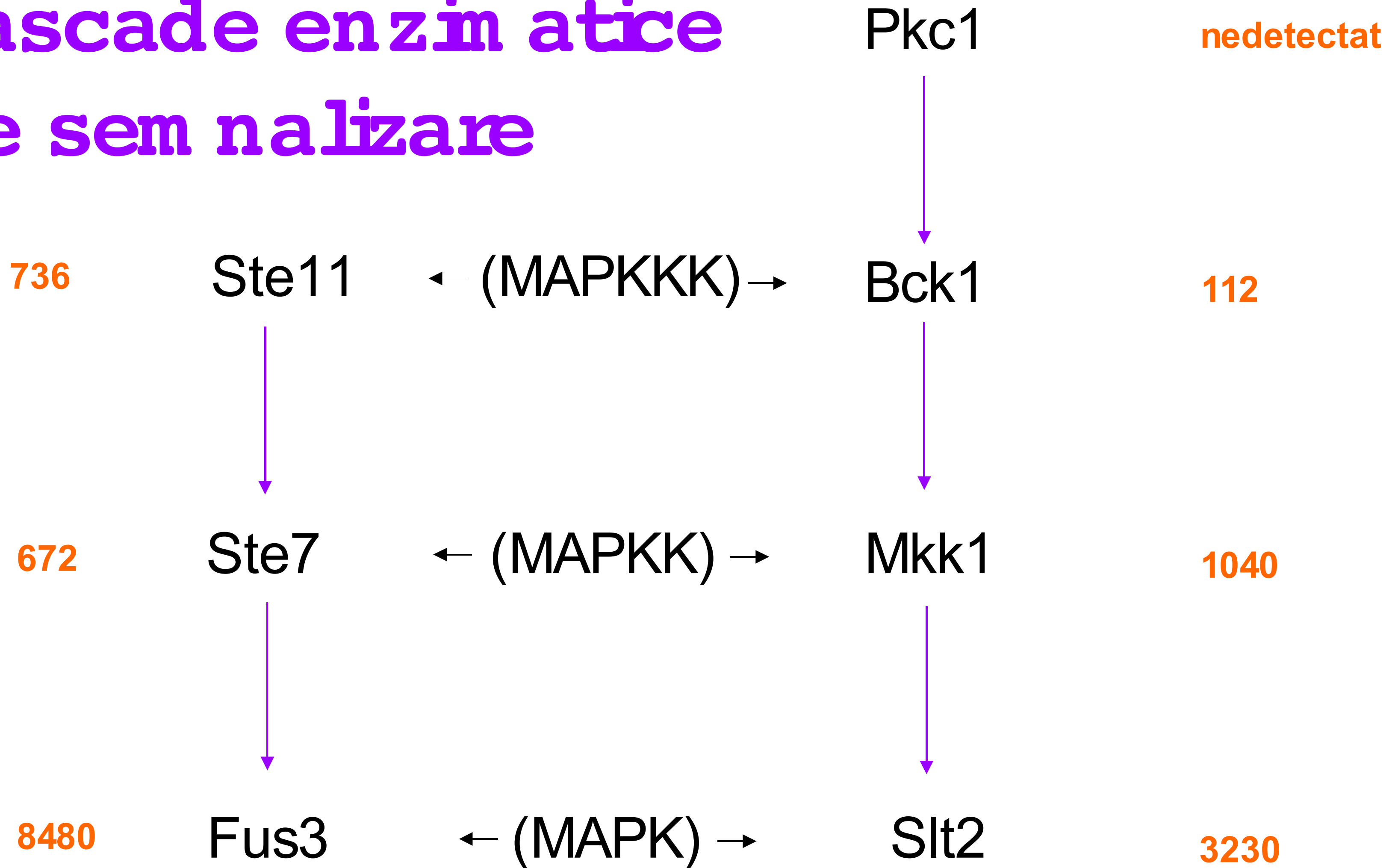
Câte molecule din enzimele glicolitice se aflã într-o celulã de drojdie?

10709 (6385)



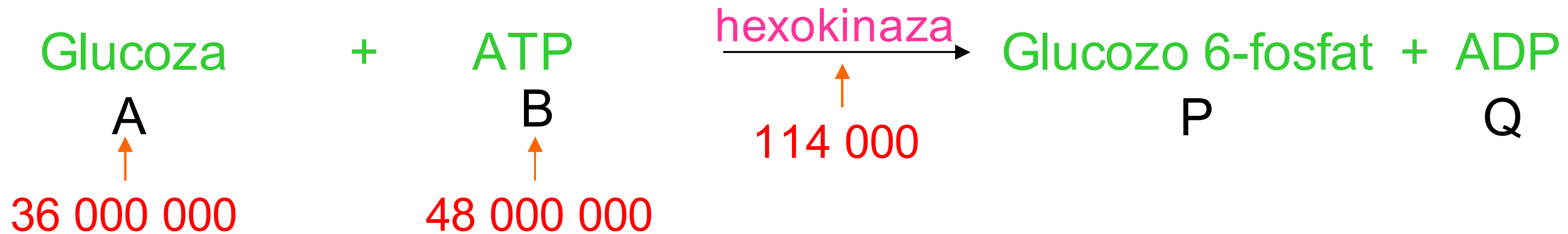
Sursa datelor numerice: Nature **441**, 840-846 (2006)

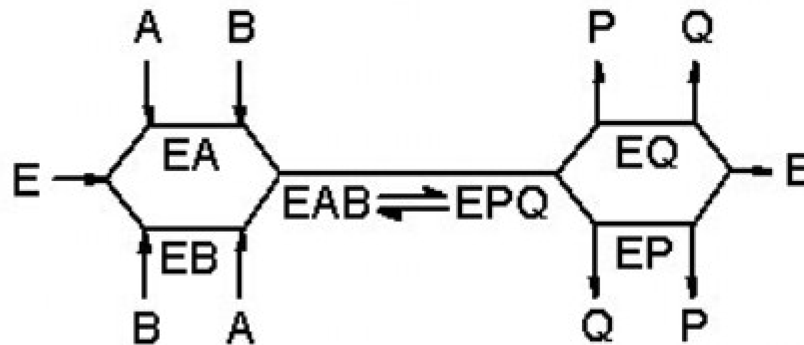
Doua exemple numerice de cascade enzimatice de semnalizare



Sursa datelor numerice: Nature **425**, 737-741 (2003)

Analiza detaliata pentru o reactie enzimatica din celula de drojdie



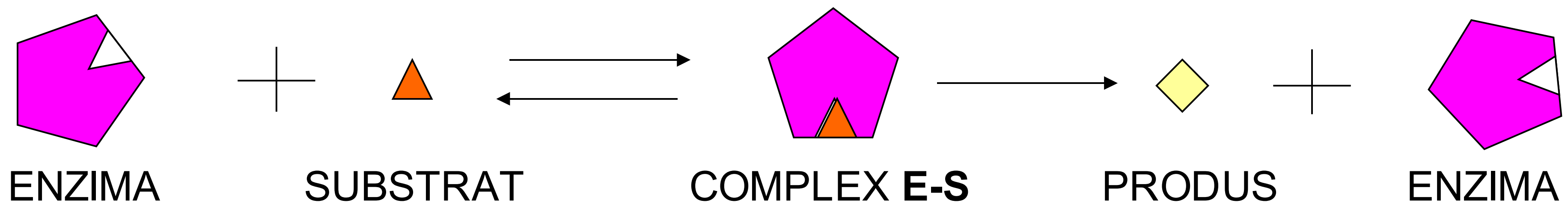


Transportori ai glucozei: > 20 tipuri
reprezentanti mai importanti:

HXT1 2330
HXT3 37200
HXT7 7350
HXT8 623

Constante macroscopice pentru hexokinaza:

$K_{m,glucoza}$ = 0.12 mM
$K_{m,ATP}$ = 0.15 mM
k_{cat} = 1.06 sec^{-1}
$K_{m\ transport\ afinitate\ scazuta}$ = 55 mM


ENZIMA
SUBSTRAT
COMPLEX E-S
PRODUS
ENZIMA

Motive de invaliditate a aplicarii legilor macroscopice la procesele enzimatice intracelulare

- numarul redus de molecule/celula pentru unele enzime conduc la alte legi cinetice decat legea actiunii maselor
- propagarea perturbatiilor pe lanturi de reactii enzimatice/ lanturi de semnalizare
- procesele de transport nu sunt procese clasice de difuzie (ex: transport activ, difuzie facilitata)
- deplasarea impiedicata a moleculelor din interior din cauza

  - densitatii mari de molecule intracelulare
  - existenta arhitecturii citoscheletale