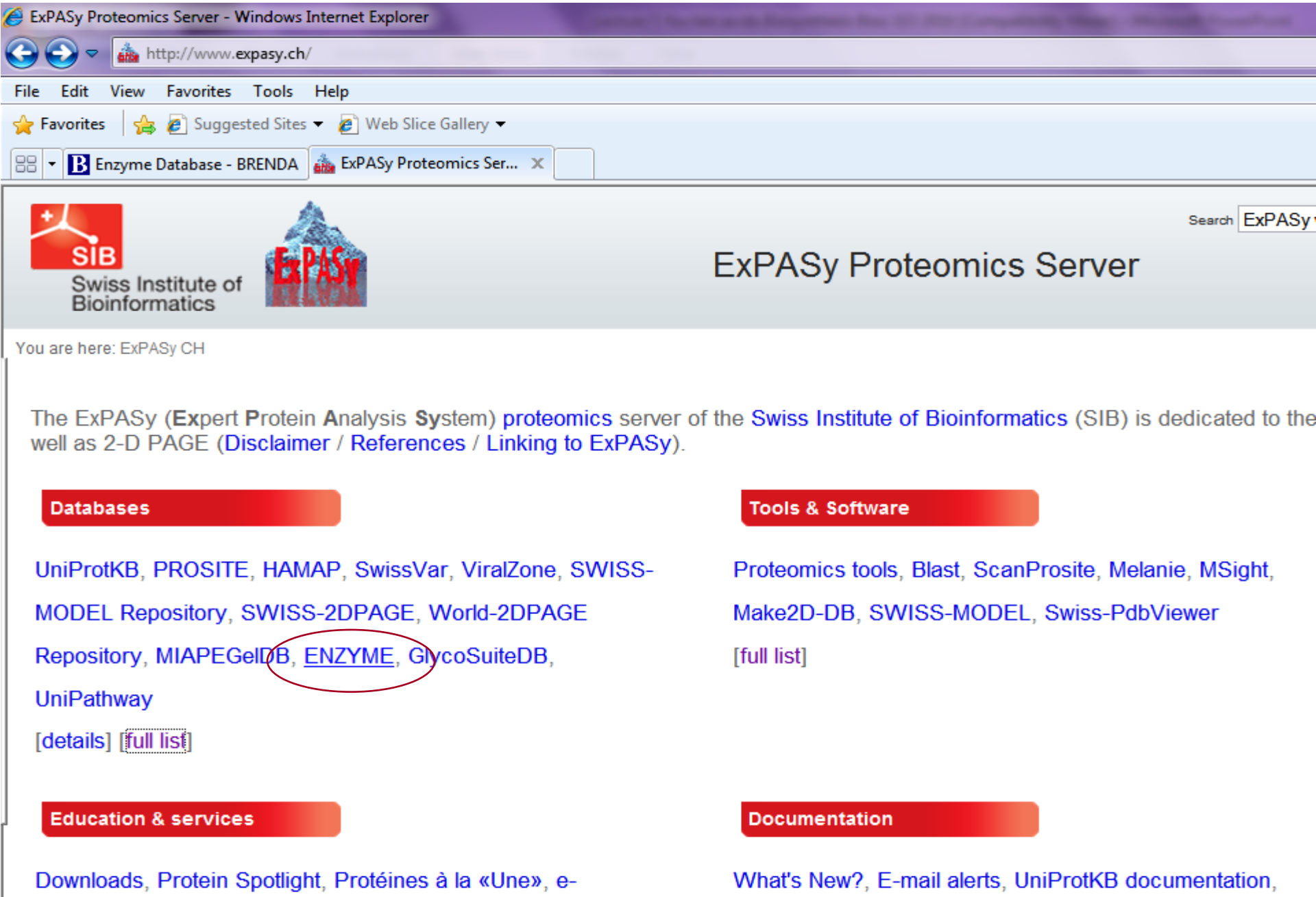
Purine biosynthesis (cont) and regulation

Link to Bioinformatics – information on proteins, eg enzymes…



ExPASy - ENZYME - Windows Internet Explorer

http://www.expasy.ch/enzyme/

File Edit View Favorites Tools Help

Favorites | Suggested Sites | Web Slice Gallery

Enzyme Database - BRENDA | ExPASy - ENZYME

Swiss Institute of Bioinformatics

Search ENZYME

ExPASy Proteomics Server

You are here: ExPASy CH > Databases > Enzyme

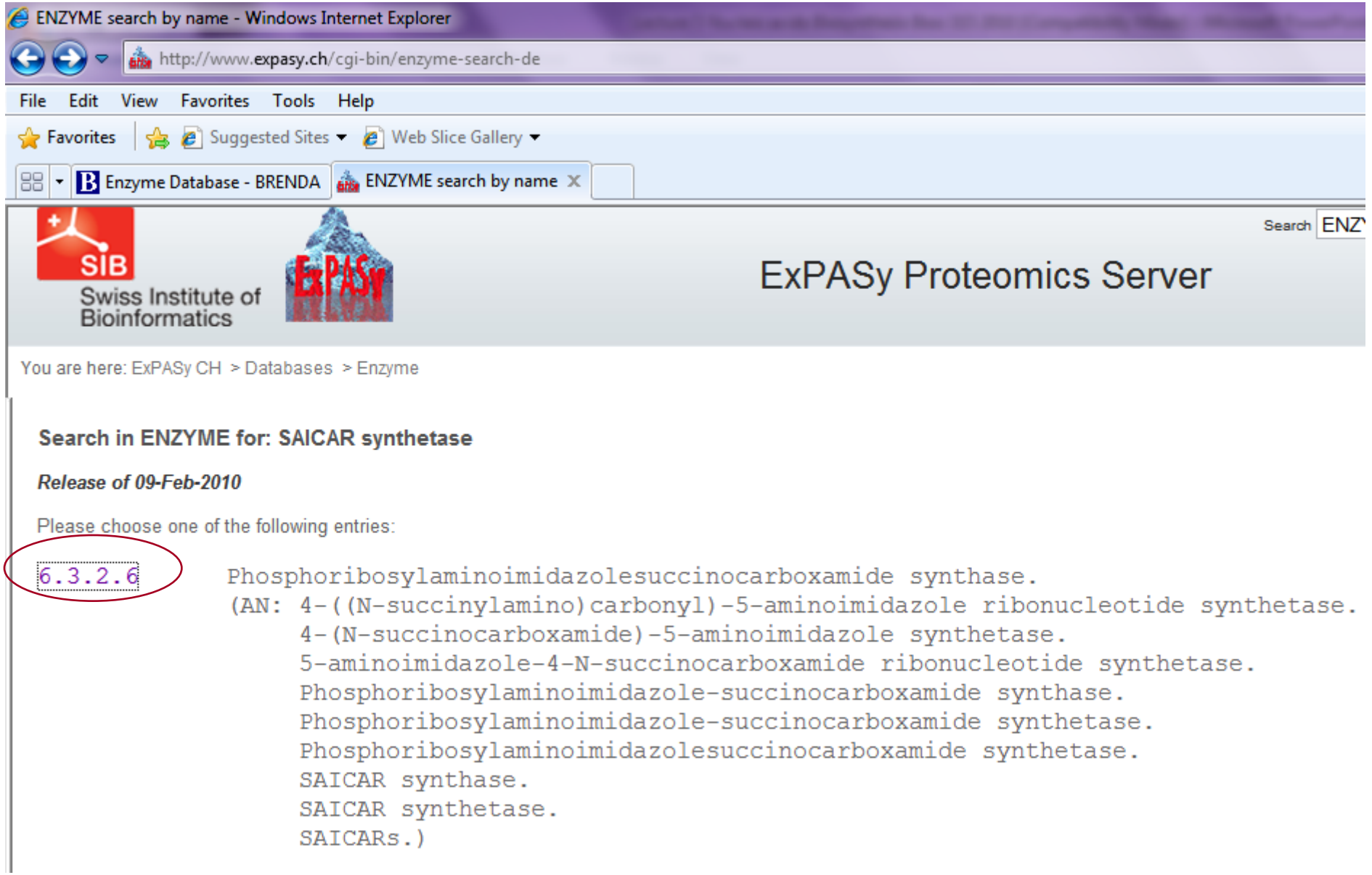
ENZYME
Enzyme nomenclature database

ENZYME is a repository of information relative to the nomenclature of enzymes. It is primarily based on the recommendations of the Nomenclature Committee of th
(IUBMB) and it describes each type of characterized enzyme for which an EC (Enzyme Commission) number has been provided [More details / References / Linkir

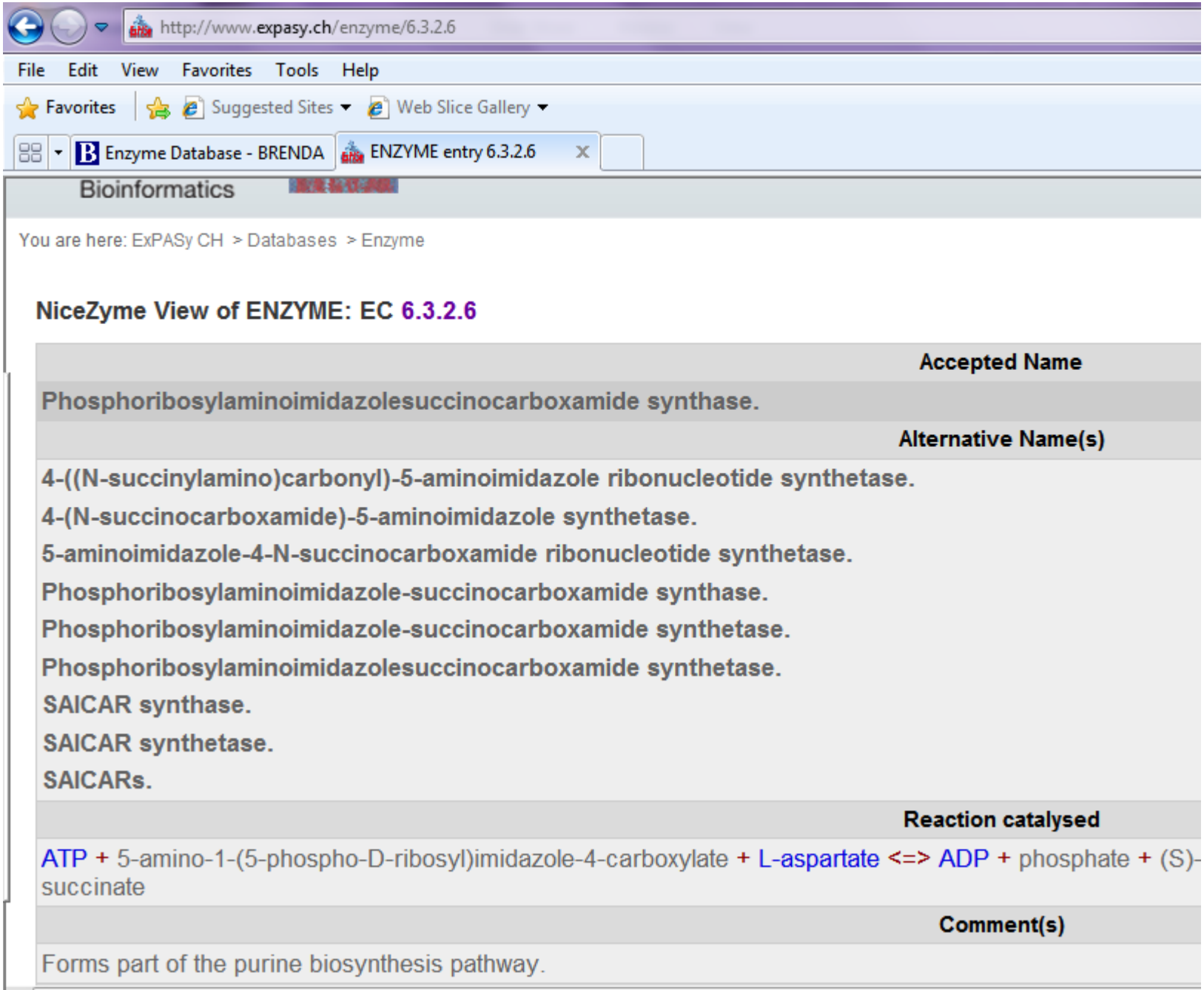
Release of 09-Feb-2010 (4154 active entries)

Access to ENZYME

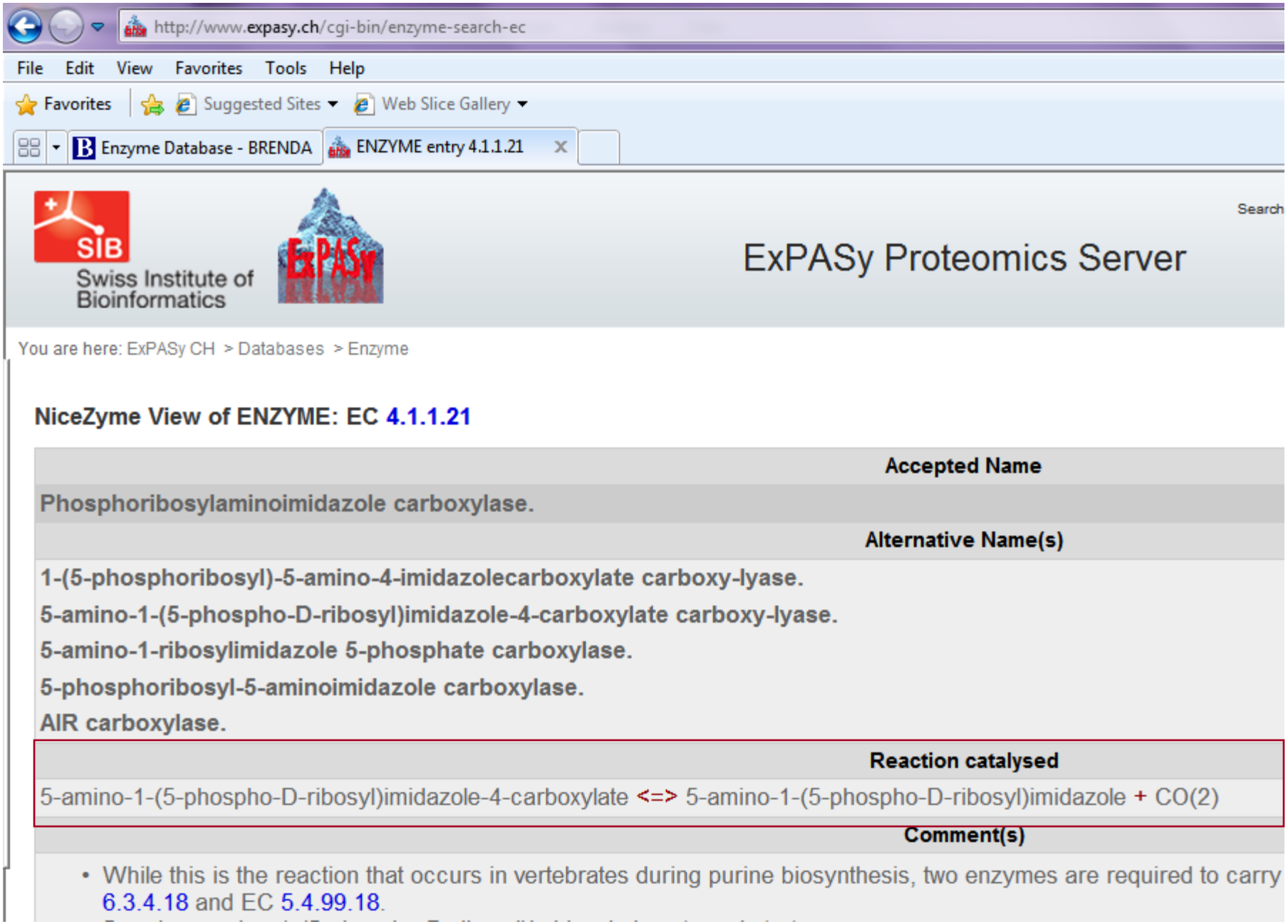
- by EC number: . . . Search
- by enzyme class
- by description (official name) or alternative name(s): SAICAR synthetase Search
- by chemical compound
- by cofactor
- by search in comments lines


ENZYME search by name - Windows Internet Explorer
http://www.expasy.ch/cgi-bin/enzyme-search-de
File Edit View Favorites Tools Help
Favorites
Suggested Sites
Web Slice Gallery
Enzyme Database - BRENDA
ENZYME search by name
Search ENZ
SIB
Swiss Institute of Bioinformatics
ExPASy
ExPASy Proteomics Server
You are here: ExPASy CH > Databases > Enzyme
Search in ENZYME for: SAICAR synthetase
Release of 09-Feb-2010
Please choose one of the following entries:
6.3.2.6 Phosphoribosylaminoimidazolesuccinocarboxamide synthase.
(AN: 4-((N-succinylamino)carbonyl)-5-aminoimidazole ribonucleotide synthetase.
4-(N-succinocarboxamide)-5-aminoimidazole synthetase.
5-aminoimidazole-4-N-succinocarboxamide ribonucleotide synthetase.
Phosphoribosylaminoimidazole-succinocarboxamide synthase.
Phosphoribosylaminoimidazole-succinocarboxamide synthetase.
Phosphoribosylaminoimidazolesuccinocarboxamide synthetase.
SAICAR synthase.
SAICAR synthetase.
SAICARs.)

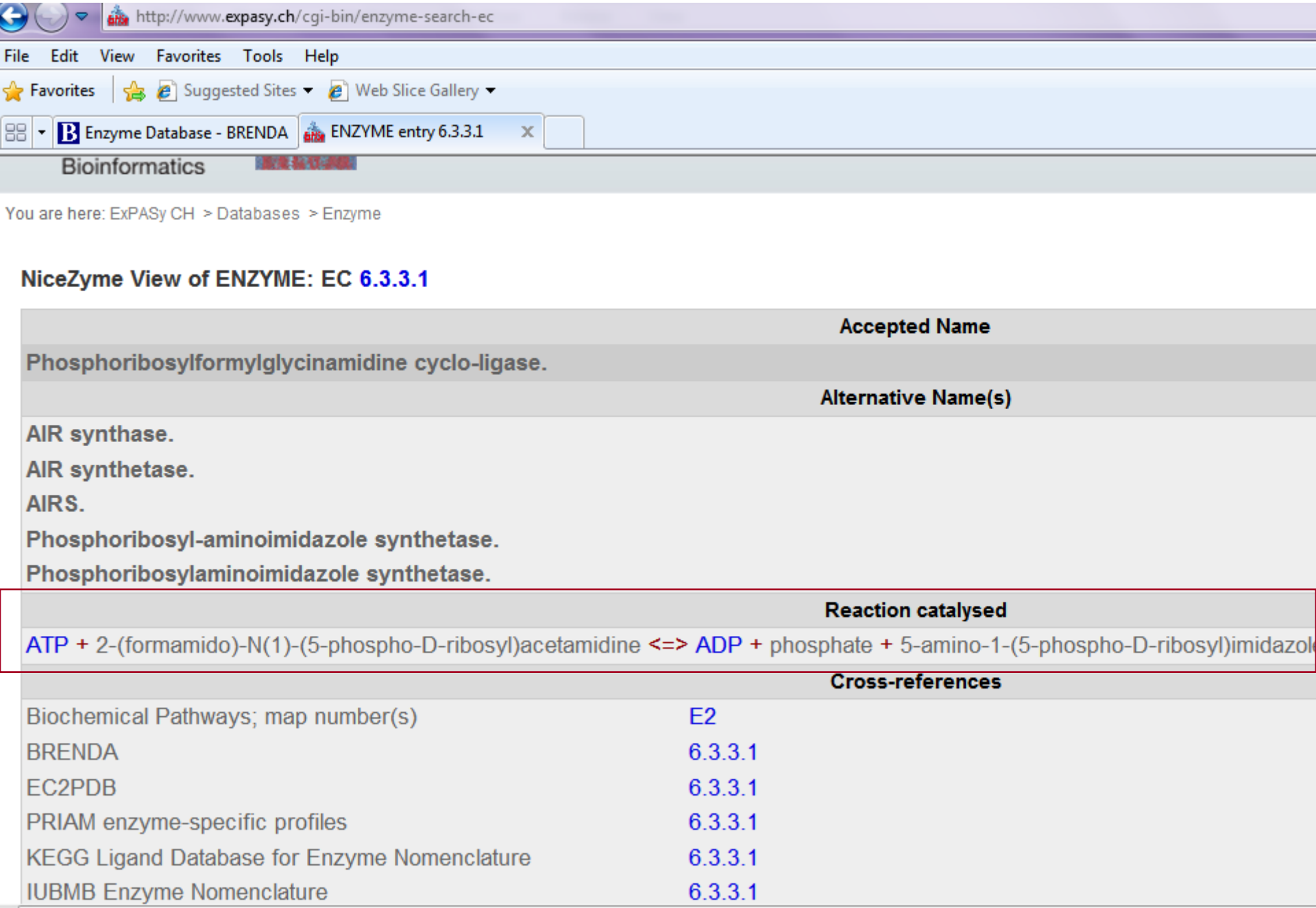
Synthetase = synthase



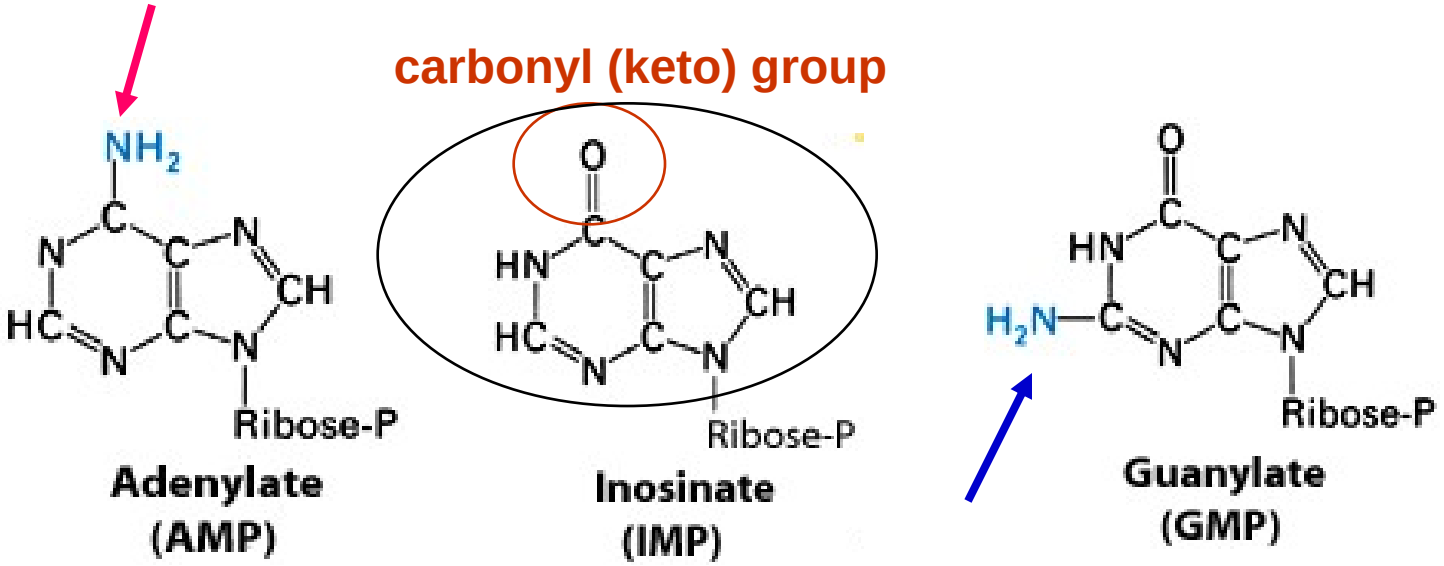
AIR carboxylase reaction (reaction 6) does not require ATP (Horton is incorrect)



AIR synthetase reaction (reaction 5) does require ATP



Chemical structure of IMP, AMP and GMP

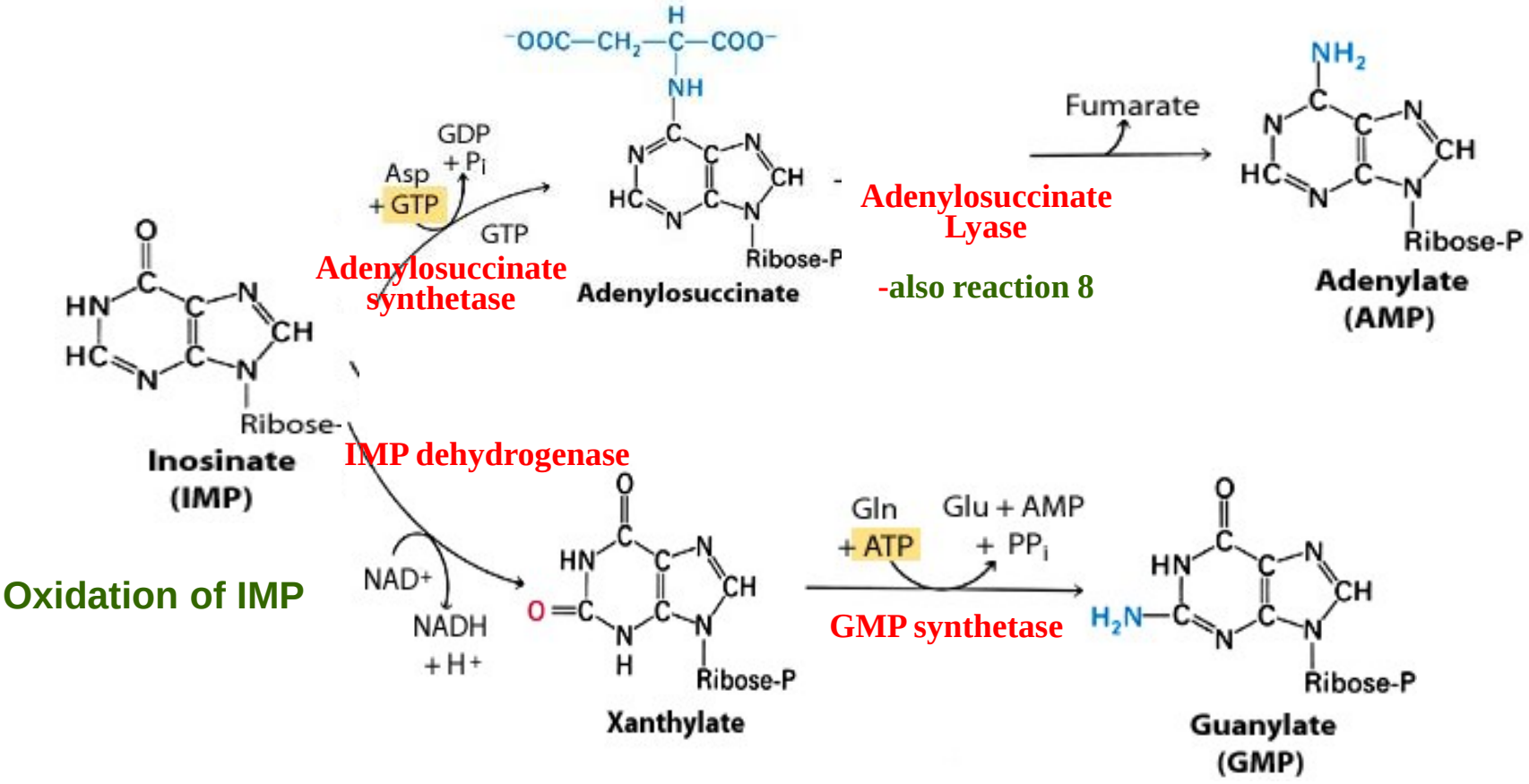


AMP differs from IMP by the C6 amino group instead of carbonyl group

GMP differs from IMP by the C2 amino group.

Base of IMP = Hypoxanthine.

Synthesis of AMP or GMP from IMP



AMP and GMP are synthesized from inosinic acid (IMP). Synthesis of AMP and GMP is a branched pathway from IMP.

The amino groups for AMP and GMP come from different sources: Asp for AMP, and Gln for GMP. Energy sources are also different (NB)

Enzyme structure

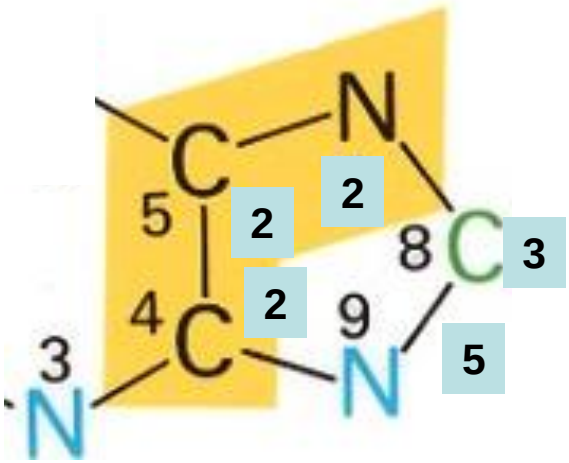
- **Multi-enzyme complex**: enzymes catalysing several of the steps are part of a single protein (multi-functional enzyme)

- In eukaryotic cells
  - enzymes catalysing steps 2, 3 and 5 are on a single protein
  - enzymes catalysing steps 6, 7 are on a single protein.
  - enzymes catalysing 9, 10 are on a single protein.

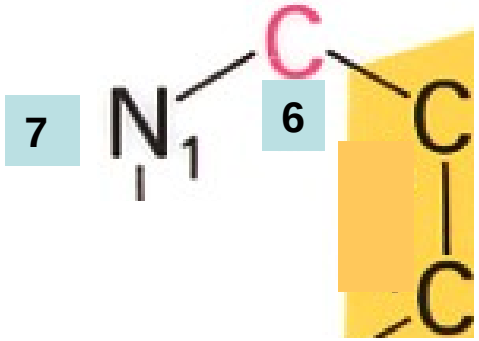
- Intermediates channelled to next enzyme
  - Increased rate
  - Decreased degradation

- In prokaryotes (bacteria) the enzymes are each on separate proteins.

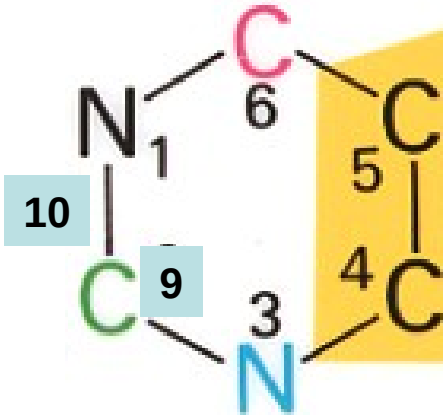
- Steps 2, 3, 5
  - Imidazole

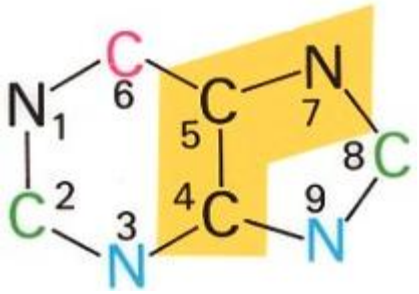


- Steps 6, 7



- Step 8, 9, 10

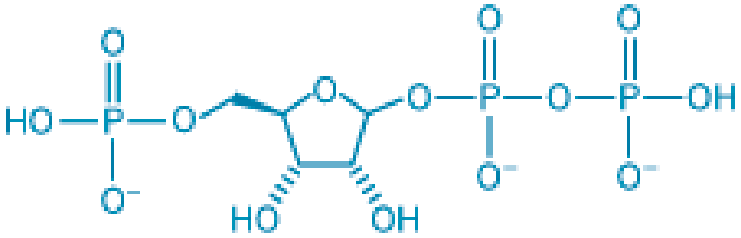




Original purine

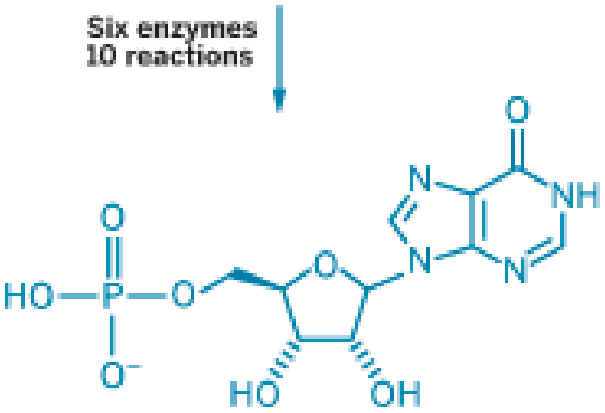
The proposed **purinosome** protein complex

converts phosphoribosylpyrophosphate (PRPP) to inosine monophosphate (IMP).



PRPP

Purinosome protein complex – consists of a 6-enzyme complex (including 3 multifunctional enzymes



IMP

http://www.sciencemag.org/cgi/reprint/320/5872/103.pdf

Reversible Compartmentalization of de Novo Purine Biosynthetic Complexes in Living Cells

Songon An,* Ravindra Kumar, Erin D. Sheets,* Stephen J. Benkovic*

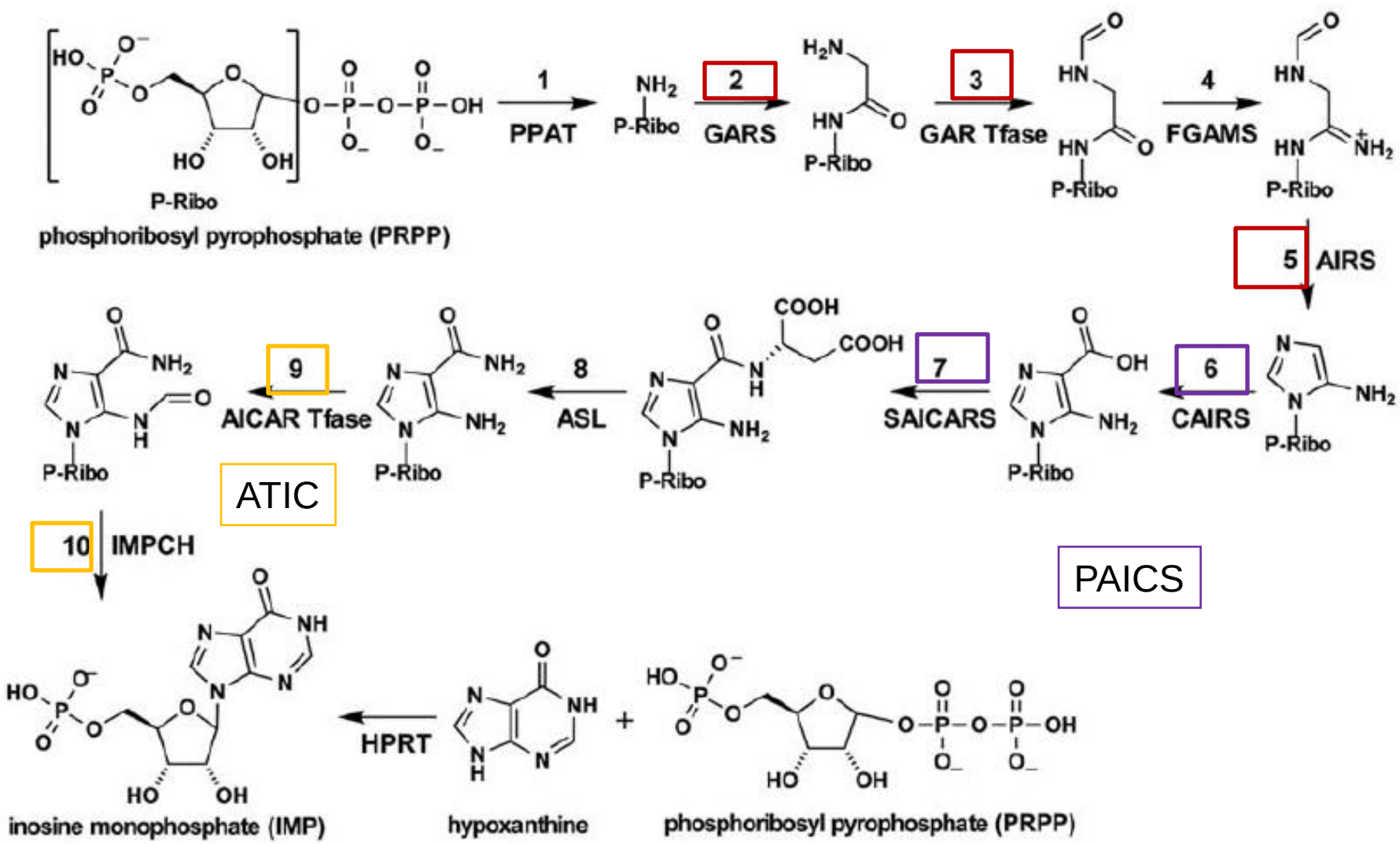
Purines are synthesized de novo in 10 chemical steps that are catalyzed by six enzymes in eukaryotes. Studies in vitro have provided little evidence of anticipated protein-protein interactions that would enable substrate channeling and regulation of the metabolic flux. We applied fluorescence microscopy to HeLa cells and discovered that all six enzymes colocalize to form clusters in the cellular cytoplasm. The association and dissociation of these enzyme clusters can be regulated dynamically, by either changing the purine levels of or adding exogenous agents to the culture media. Collectively, the data provide strong evidence for the formation of a multi-enzyme complex, the "purinosome," to carry out de novo purine biosynthesis in cells.

Department of Chemistry, The Pennsylvania State University, University Park, PA 16802, USA.

*To whom correspondence should be addressed. E-mail: sjb1@psu.edu (S.J.B.); eds11@psu.edu (E.D.S.); sua13@psu.edu (S.A.)

SCIENCE VOL 320 4 APRIL 2008

Purines are not only essential building blocks of DNA and RNA, but, as nucleotide derivatives, they also participate in a multitude of pathways in both prokaryotes and eukaryotes (*1*, *2*). Biosynthetically, adenosine and guanosine nucleotides are derived from inosine monophosphate (IMP), which is synthesized from phosphoribosyl pyrophosphate (PRPP) in both the de novo and salvage biosynthetic pathways (Fig. 1). The salvage pathway catalyzes the one-step conversion of hypoxanthine to IMP by hypoxanthine phosphoribosyl transferase (HPRT), whereas the de novo pathway consists of 10 chemical reactions that transform PRPP to IMP. In higher eukaryotes (such as humans), the de novo pathway uses six enzymes, including three multifunctional enzymes: a trifunctional protein, TrifGART, that has glycinamide ribonucleotide (GAR) synthetase (GARS) (step 2), GAR transformylase (GAR Tfase) (step 3), and aminoimidazole ribonucleotide synthetase (AIRS) (step 5) activities; a bifunctional enzyme, PAICS, that has carboxyaminoimidazole ribonucleotide synthase (CAIRS) (step 6) and succinylaminoimidazolecarboxamide ribonucleotide synthetase (SAICARS) (step 7) activities; and a bifunctional enzyme, ATIC, that has aminoimidazolecarboxamide ribonucleotide transformylase (AICAR Tfase) (step 9) and IMP cyclohydrolase (IMPCH) (step 10) activities. In contrast, prokaryotes, such as *Escherichia coli*, use only monofunctional enzymes throughout this pathway, except for the bifunctional ATIC.


TrifGART
phosphoribosyl pyrophosphate (PRPP)
1
PPAT
2
GARS
3
GAR Tfase
4
FGAMS
5 AIRS
6
CAIRS
7
SAICARS
8
ASL
9
AICAR Tfase
ATIC
10 IMPCH
PAICS
HPRT
inosine monophosphate (IMP)
hypoxanthine
phosphoribosyl pyrophosphate (PRPP)

Although studies of the individual enzymes in vitro have revealed much about their respective mechanisms of action, a number of in vitro attempts using kinetic analysis and/or binding measurements to demonstrate protein-protein interactions have not been fruitful, with few exceptions (*3*–*6*). In addition, there is scant evidence from in vivo cellular studies for the hypothesis that these enzymes act in concert within a multienzyme complex (*7*). In this study, we used fluorescence microscopy to investigate whether functional multienzyme complexes involved in de novo purine biosynthesis form in living mammalian cells under purine-rich and purine-depleted conditions, which affect the rate of metabolic flux (*8*–*10*).

Mechanism of action is known (how they convert their substrate to product)

Protein-protein interaction as multi-enzyme complex is unclear

Ref 7: work done in cells; in vivo for bacteria, but not strictly in vivo in mammalian cells

Method

Fluorescence microscopy
Cell culture (purine-rich vs –depleted)
Human TrifGART – GFP (step 2,3,5)
Human FGAMS – GFP or OFP (step 4)
Hela cells

We selected for study two human (h) enzymes involved with de novo purine biosynthesis as initial candidates for involvement in a multienzyme complex in vivo: the nonsequential hTrifGART protein, which catalyzes steps 2, 3, and 5, and formylglycinamidine ribonucleotide synthase (hFGAMS), which catalyzes step 4. These proteins were fused to either a green fluorescent protein (GFP) or an orange fluorescent protein (OFP) and were transiently expressed in HeLa cells that had been cultured in either purine-rich [minimum essential medium and 10% fetal bovine serum (FBS)] or purine-depleted (RPMI 1640 and dialyzed 5% FBS) media (*9*, *11*).

Reference 7 of purinosome paper

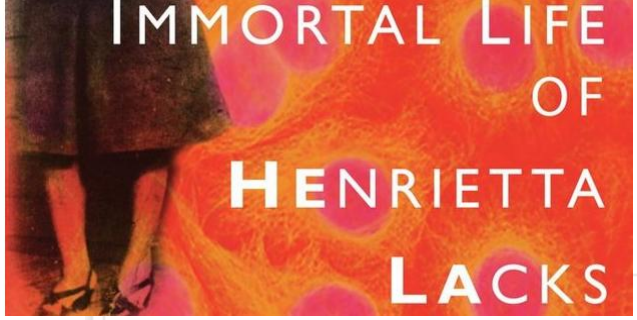
Localization of GAR transformylase in *Escherichia coli* and mammalian cells

Lata T. Gooljarsingh*†, Joseph Ramcharan*†, Simon Gilroy‡, and Stephen J. Benkovic*§

*Department of Chemistry, 414 Wartik Laboratory, and ‡Department of Biology, 208 Mueller Laboratory, Pennsylvania State University, University Park, PA 16802

Contributed by Stephen J. Benkovic, April 12, 2001

Enzymes of the *de novo* purine biosynthetic pathway may form a multienzyme complex to facilitate substrate flux through the ten serial steps constituting the pathway. One likely strategy for complex formation is the use of a structural scaffold such as the cytoskeletal network or subcellular membrane of the cell to mediate protein–protein interactions. To ascertain whether this strategy pertains to the *de novo* purine enzymes, the localization pattern of the third purine enzyme, glycinamide ribonucleotide transformylase (GAR Tfase) was monitored in live *Escherichia coli* and mammalian cells. Genes encoding human as well as *E. coli* GAR Tfase fused with green fluorescent protein (GFP) were introduced into their respective cells with regulated expression of proteins and localization patterns monitored by using confocal fluorescence microscopy. In both instances images showed proteins to be diffused throughout the cytoplasm. Thus, GAR Tfase is not localized to an existing cellular architecture, so this device is probably not used to concentrate the members of the pathway. However, discrete clusters of the pathway may still exist throughout the cytoplasm.



Rebecca Skloot, author of *The Immortal Life Of Henrietta Lacks*

About *The Immortal Life of Henrietta Lacks*

Her name was Henrietta Lacks, but scientists know her as HeLa. She was a poor black tobacco farmer whose cells—taken without her knowledge in 1951—became one of the most important tools in medicine, vital for developing the polio vaccine, cloning, gene mapping, in vitro fertilization, and more. Henrietta's cells have been bought and sold by the billions, yet she remains virtually unknown, and her family can't afford health insurance.

Soon to be made into an HBO movie by Oprah Winfrey and Alan Ball, this *New York Times* bestseller takes readers on an extraordinary journey, from the "colored" ward of Johns Hopkins Hospital in the 1950s to stark white laboratories with freezers filled with HeLa cells, from Henrietta's small, dying hometown of Clover, Virginia, to East Baltimore today, where her children and grandchildren live and struggle with the legacy of her cells. *The Immortal Life of Henrietta Lacks* tells a riveting story of the collision between ethics, race, and medicine; of scientific discovery and faith healing; and of a daughter consumed with questions about the mother she never knew. It's a story inextricably connected to the dark history of experimentation on African Americans, the birth of bioethics, and the legal battles over whether we control the stuff we're made of.

The Henrietta Lacks Foundation

The Henrietta Lacks Foundation strives to provide financial assistance to needy individuals who have made important contributions to scientific research without their knowledge or consent.

When expressed individually in cells grown in purine-rich media, both the hTrifGART (Fig. 2A) and hFGAMS (Fig. 2C) proteins exhibited diffuse cytoplasmic distributions, as previously found in 293T fibroblast cells and with their prokaryotic counterpart, *E. coli* cells (*7*). However, when these constructs were expressed in purine-depleted cells, we observed cytoplasmic clustering, estimated at ~27% of the hTrifGART-GFP (Fig. 2B) and ~77% of the hFGAMS-GFP (Fig. 2D) proteins. To determine whether the two enzymes colocalized into these clusters in low-purine conditions, we transiently coexpressed hTrifGART-GFP and hFGAMS-OFP in HeLa cells (Fig. 2, G, H, and I); ~60% of the transfected cells exhibited colocalization within the clusters (Fig. 2I).

of the hFGAMS-GFP (Fig. 2D) proteins. To determine whether the two enzymes colocalized into these clusters in low-purine conditions, we transiently coexpressed hTrifGART-GFP and hFGAMS-OFP in HeLa cells (Fig. 2, G, H, and I); ~60% of the transfected cells exhibited colocalization within the clusters (Fig. 2I).

No Clusters ⇓ Purine-rich

Clusters ⇓ Purine-depleted

hTrifGART -GFP

Hfgams - GFP

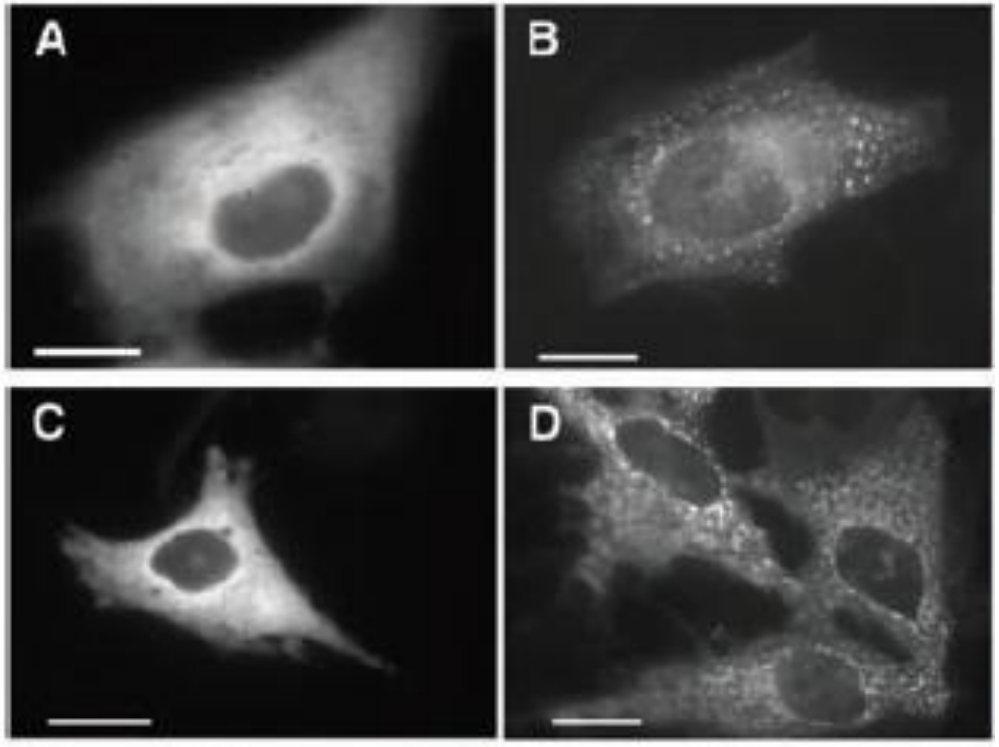
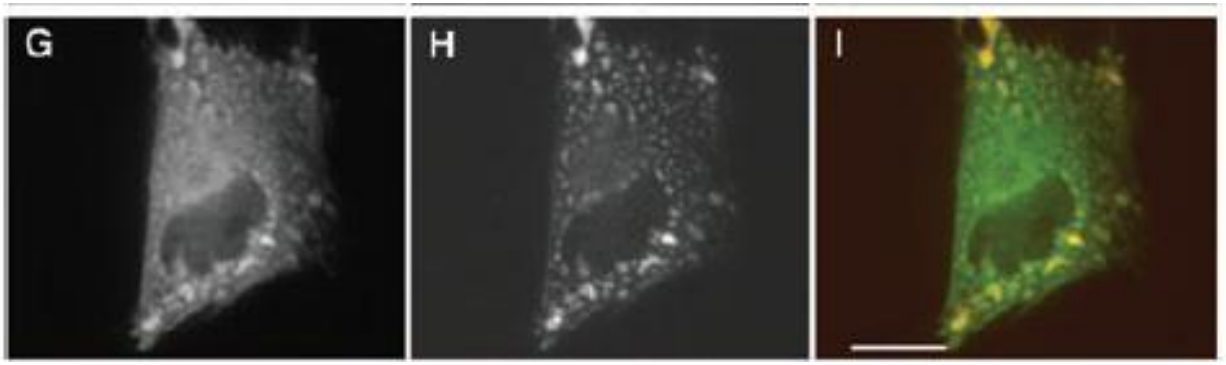


Fig 2

Co-localisation:

hTrifGart-GFP = green
hFGAMS-OFP = orange
Co-localise = yellow

hTrifGart-GFP **hFGAMS-OFP**

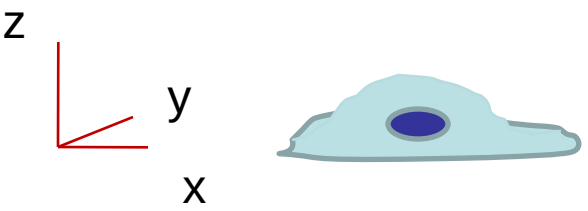


Step 2,3,5
and
Step 4

Fig 2

Z-scan imaging with confocal laser-scanning microscopy confirmed that the hTrifGART-GFP and the hFGAMS-OFP proteins were co-clustered in the cytoplasm (fig. S1). We also observed co-clustering between hFGAMS-GFP and hTrifGART-OFP after we reversed the fusion of the fluorescent probes in HeLa cells that were maintained in purine-depleted media (fig. S2). Additionally, similar cellular localization experiments were carried out with hTrifGART-GFP and hFGAMS-GFP in two additional human cell lines, HTB-125 and HTB-126; the results suggested that the purine-dependent clustering appears to occur in other human cell lines [supporting online material (SOM) Text]. These findings, when combined with the imaging results described

for HeLa cells, suggest that the formation of this multienzyme complex involved in the de novo purine biosynthetic pathway may be functionally relevant in mammalian cells, particularly those challenged with minimal media.

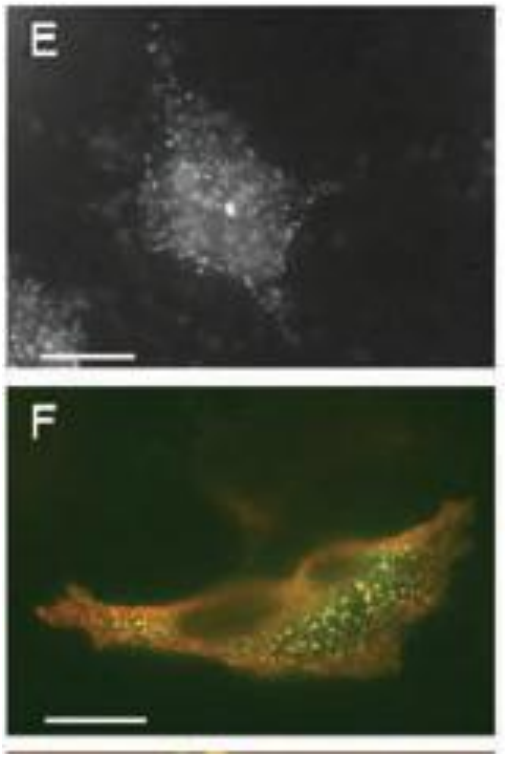


--Same co-localisation if you switch the fluorescent probes
--Same co-localisation in other cells

To verify that the clustering we observed was not due to an artifact of expressing the fluorescent fusion constructs but was in fact present with endogenously expressed protein, we conducted immunofluorescence imaging of endogenous hTrifGART protein in HeLa cells grown in purine-depleted media; clustering was clearly observed (Fig. 2E), similar to what we found for the hTrifGART-GFP protein under the same conditions (Fig. 2B). We also identified some clustering of endogenous hTrifGART in the presence of purine-rich media (fig. S3), which we attribute either to the sensitivity of immunofluorescence in detecting protein expression or to the loss of cytosolic materials during fixation. We further demonstrated that endogenous hTrifGART co-clustered with hTrifGART-GFP in HeLa cells that were maintained in purine-depleted media (Fig. 2F), which supports our initial observations with the GFP fusion constructs. Taken together, these results suggest that multienzyme complexes form to satisfy the cellular demand for de novo purine biosynthesis.

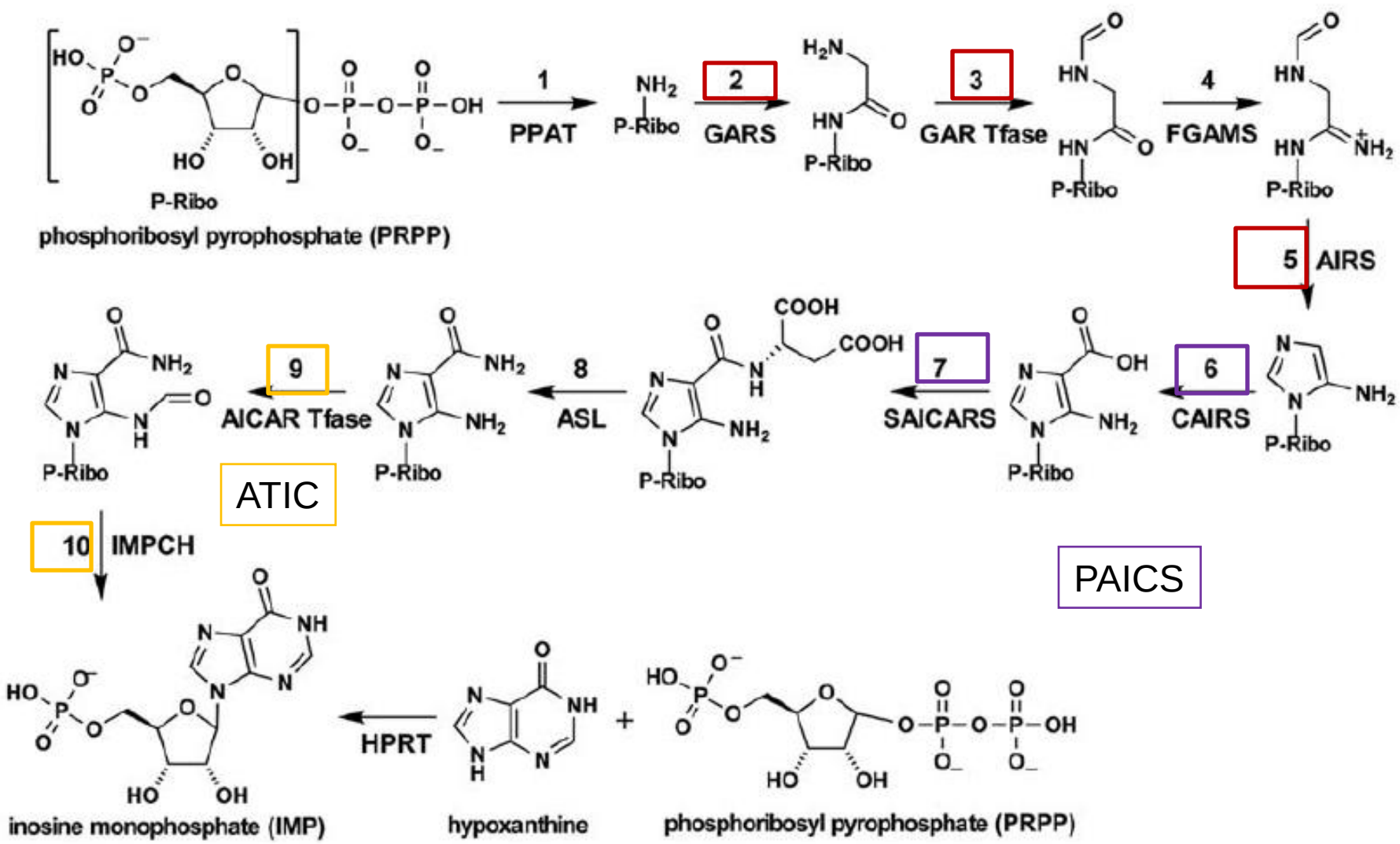
Background?

Endogenous hTrifGART



Endogenous hTrifGART = red
hTrifGart-GFP = green
Co-localise = yellow

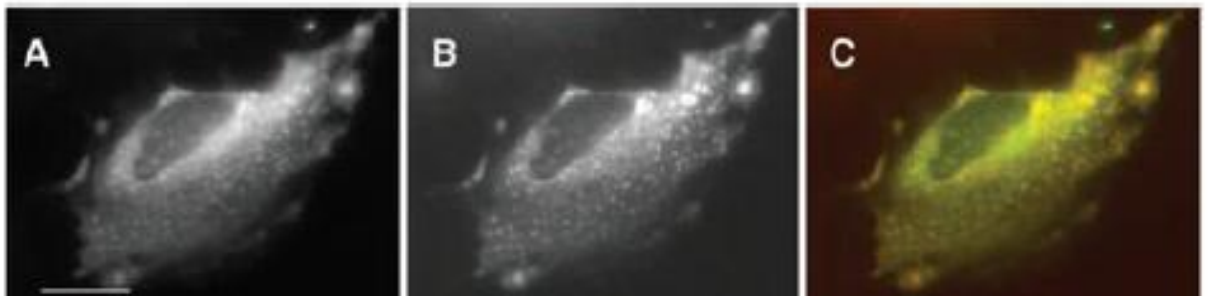
Fig 2


TrifGART
phosphoribosyl pyrophosphate (PRPP)
1
PPAT
2
GARS
3
GAR Tfase
4
FGAMS
5 AIRS
6
CAIRS
7
SAICARS
8
ASL
9
AICAR Tfase
ATIC
10 IMPCH
PAICS
HPRT
inosine monophosphate (IMP)
hypoxanthine
phosphoribosyl pyrophosphate (PRPP)

We expanded our studies to include all six human enzymes of the pathway (Fig. 1): PRPP amidotransferase (hPPAT) (step 1), hTrifGART, hFGAMS, hPAICS, adenylosuccinate lyase (hASL) (step 8), and hATIC. As a control, we also included a tetrahydrofolate (H_4F)–utilizing trifunctional enzyme, hC1THF, that possesses *5,10*-methylene-H_4F dehydrogenase, *5,10*-methenyl-H_4F cyclohydrolase, and *10*-formyl-H_4F synthetase activities. hC1THF is not directly involved in the de novo purine pathway but is responsible for synthesizing a key cofactor, *10*-formyl-H_4F, for both hTrifGART and hATIC activities (*5*, *12*). We found that all six enzymes that directly participate in the de novo purine biosynthetic pathway co-clustered with hFGAMS-OFP in the cytoplasm of cells maintained in purine-depleted media (Fig. 2, G to I, and Fig. 3); however, hC1THF-GFP was diffuse throughout the cytoplasm, and thus did not co-cluster with hFGAMS-OFP (Fig. 2, J to L).

hPPAT-GFP = green
hFGAMS-OFP = orange
Co-localise = yellow

hPPAT-GFP **hFGAMS-OFP**



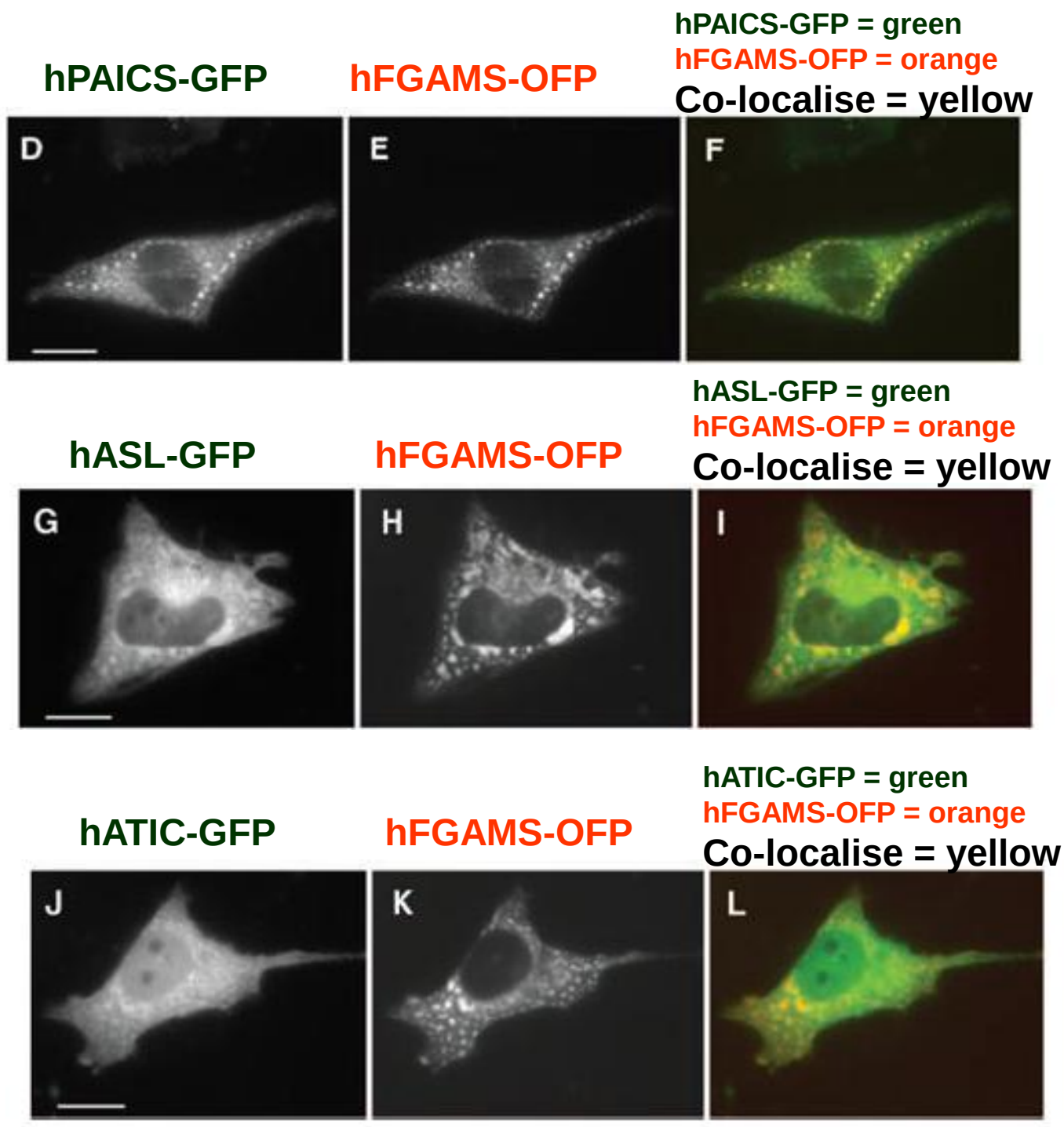
Step 1
and
Step 4

Fig 3

Did the same for:

Step6/7 and Step 4
Step 8 and Step 4
Step 9/10 and Step 4

Fig 3


hPAICS-GFP
hFGAMS-OFP
hPAICS-GFP = green
hFGAMS-OFP = orange
Co-localise = yellow
D
E
F
Step 6/7
and
Step 4
hASL-GFP
hFGAMS-OFP
hASL-GFP = green
hFGAMS-OFP = orange
Co-localise = yellow
G
H
I
Step 8
and
Step 4
hATIC-GFP
hFGAMS-OFP
hATIC-GFP = green
hFGAMS-OFP = orange
Co-localise = yellow
J
K
L
Step 9/10
and
Step 4

Cluster formation is specific to enzymes of purine biosynthesis pathway

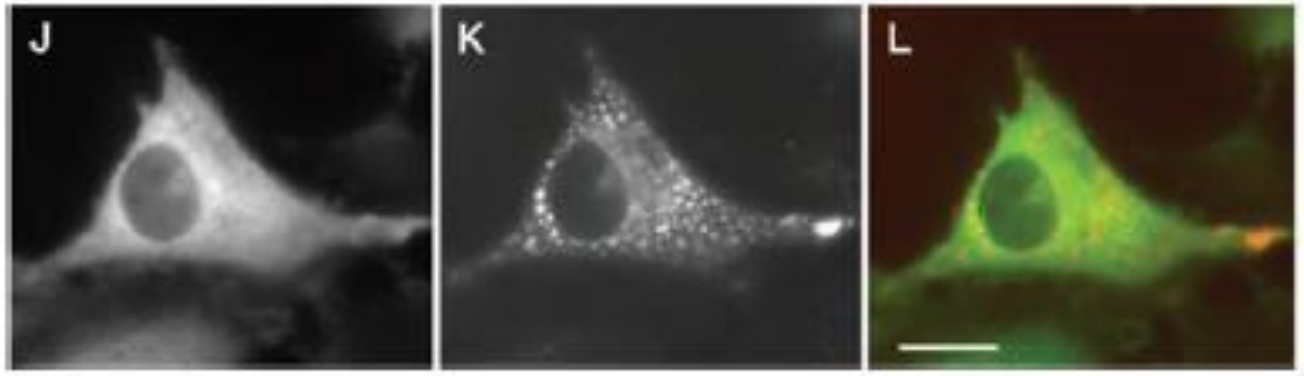
Colocalization analysis collectively revealed that 78 ± 6% of GFP-fusion proteins co-clustered with hFGAMS-OFP. However, the analysis for hC1THF-GFP and hFGAMS-OFP exhibited 21 ± 5% colocalization, which suggests that these proteins do not colocalize in clusters: The representative image (Fig. 2L) clearly shows hFGAMS-OFP clusters on a diffuse background of hC1THF-GFP.

No colocalisation

Control enzyme-GFP
hC1THF-GFP

hFGAMS-OFP

Control enzyme-GFP
hFGAMS-OFP



Percentage cluster formation increases upon co-transfection:
Eg. hTrifGART alone: 27%
TrifGART + hFGAMS: 60%

When the individual fluorescent protein constructs were transiently expressed in purine-depleted HeLa cells, the frequency of clustering was generally very low: ~5 to 8% for hPPAT-GFP, hPAICS-GFP, hASL-GFP, and GFP-hATIC, but ~27% for hTrifGART-GFP and ~77% for hFGAMS-GFP. However, when co-transfected with hFGAMS-OFP under these conditions, the clustering frequency increased substantially to ~2 to 32% for hPPAT-GFP, hPAICS-GFP, hASL-GF and GFP-hATIC, and ~60% for hTrifGART-GF The percentage of colocalization of GFP-fusion proteins with hFGAMS-OFP was calculated by dividing the number of pixels that showed both green and red colors by the total number of pixels that showed either green or red colors (*11*, *13*).

Percentage cluster formation is variable – this may be due to un-synchronised population (cell cycle)

thesis (table S1 and fig. S4). The varying percentage of cells exhibiting clustering under low-purine conditions may be attributed to cells not being synchronized with respect to the cell cycle. The G_1 and S phases require more purines to support RNA and DNA synthesis than the other phases of the cell cycle (*14*); thus, we speculate that the population of the cells exhibiting clustering may represent cells in the G_1 or S phases. This rationale is

Method of quantifying colocalisation

Cluster formation is reversible

To assess the reversibility of the multienzyme complexes, we imaged the cellular distribution of hFGAMS-GFP while purine-depleted medium was exchanged for purine-rich medium, and observed that the hFGAMS-GFP clusters dispersed within ~0.5 to 1.5 hours of HeLa cells being incubated with purine-rich medium (Fig. 4, A and B). When purine-depleted medium was subsequently restored, clusters began to re-form in ~1 to 2 hours (Fig. 4, B and C). Additionally, we performed

Step 4

Purine-depleted → Purine-rich → Purine-depleted

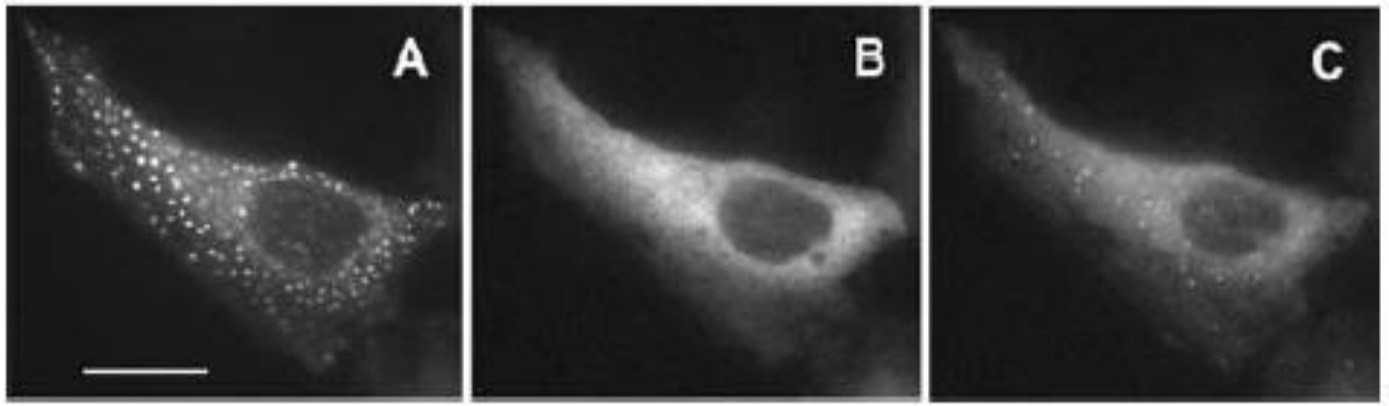


Fig. 4. Reversible formation of clusters by hFGAMS-GFP. (**A**) hFGAMS-GFP clusters when HeLa cells are exposed to purine-depleted medium. (**B**) Clusters disperse within ~0.5 to 1.5 hours upon incubation with purine-rich medium. (**C**) The clusters begin to re-form within ~1 to 2 hours after purine-depleted medium is restored. Scale bar, 10 μm.

Substrate channels?

From our studies, the various roles that the multienzyme complex plays in cells are not yet clear. These functional complexes may produce efficient substrate channels that link the 10 catalytic active sites. To date, extensive kinetic analyses of *E. coli* PPAT and GARS (*6*), *Acetobacter aceti* purE and purK (bacterial counterparts of CAIRS) (*3*), and recent crystal structures of octameric hPAICS (*18*) have revealed putative substrate channels for individual steps in the de novo pathway. Additionally, clustering of the 10 active sites may provide an efficient means of globally regulating purine flux under varying environmental conditions. However, we have not yet identified a specific subcellular compartment with which these multienzyme complexes associate, in contrast to, for example, the actin cytoskeletal association observed for aldolase (*19*). However, our cross-linking experiments with formaldehyde on living cells grown in purine-depleted media, and subsequent Western blot analysis, revealed that endogenous hTrifGART proteins produced a cross-linked adduct of molecular weight greater than 500 kDa (fig. S7), which suggests that hTrifGART is indeed compartmentalized in close proximity with other proteins, whose identities are under investigation.

Step 1, 2
Step 6

Step6/7

Global regulation of purine flux; but no specific compartment identified as yet

Cross-linking experiments show hTrifGart in a 500 kDa complex, suggestions compartmentalisation near other proteins

Purinsome: association (or clustering) of enzymes in the denovo purine biosynthesis pathway (can be regulated)

Although the association of metabolic enzymes has been claimed for enzymes involved in the glycolytic pathway (*21*, *22*), to our knowledge this complex, the glycosome, has not been identified in living mammalian cells. By analogy, the present clusters observed in the de novo purine biosynthetic pathway may constitute a "purinosome." The formation of the purinosome appears to be dynamically regulated by stimulation of de novo purine biosynthesis in response to changes in purine levels. The purinosome may be a general phenomenon in all cell types during specific stages of the cell cycle, along with posttranslation modifications. Because of the relevance of de novo purine biosynthesis to human diseases, the purinosome may represent a new pharmacological opportunity for therapeutic intervention.

Glycosome

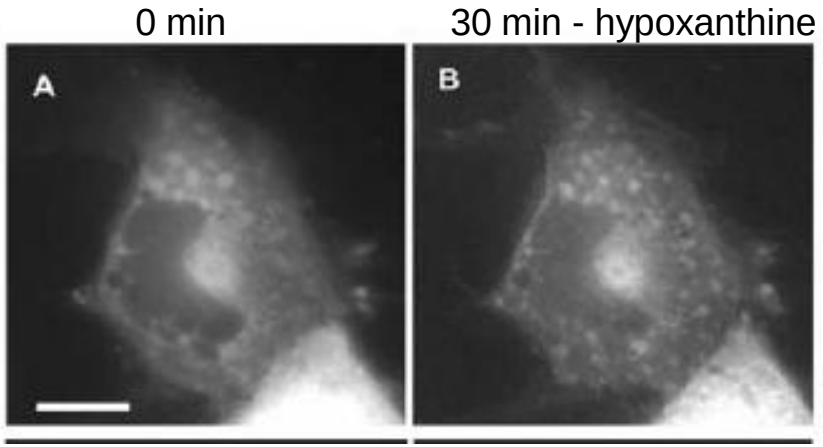
Purinosome

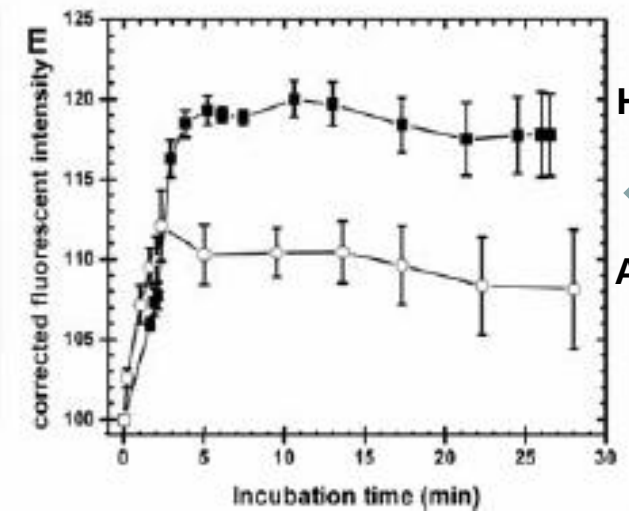
Furthermore, we imaged the effects of introducing either an exogenous purine source, hypoxanthine, or a well-known antagonist of hFGAMS, azaserine, to inhibit the metabolic flux in the de novo pathway of HeLa cells grown under purine-depleted conditions (*8*). Hypoxanthine, when added to fibroblast cultures in concentrations as low as 1 to 10 μM, significantly inhibits (>50%) de novo purine biosynthesis through its effect on adenosine and guanosine nucleotides in the cellular pools (*16*, *17*), and the increased levels of adenosine and guanosine nucleotides modulate activities of PPAT as well as PRPP synthetase (*8*). In addition, cells grown in purine-depleted media are deficient in adenosine 5′-triphosphate, with both the restoration of cellular growth rates and the timing of the G_1-to-S transition dependent on both the de novo and salvage pathways (*14*). We propose that the initial increased clustering is a response to hypoxanthine-induced inhibition of the de novo pathway and that the retention of the clusters reflects demand for purines in cellular growth (fig. S6, A, B, and solid squares in E). When we incubated azaserine with

Regulation: effects not clear

Hypoxanthine +PRPP → IMP: salvage pathway
→ Causes decreased denovo synthesis

Purine-depleted conditions





Hypoxanthine

Azaserine

Controls?

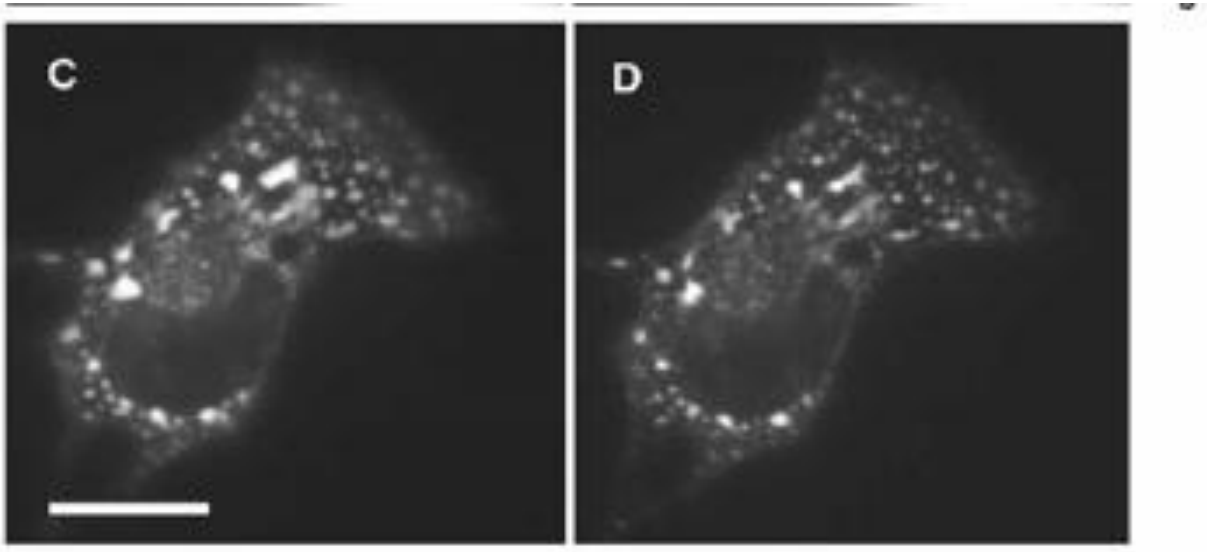
squares in E). When we incubated azaserine with purine-depleted cells, we anticipated that hFGAMS clusters would at least be maintained because of the greater demand for purine biosynthesis. An azaserine block increases the rate of the early steps of de novo purine biosynthesis in fibroblasts that lack HPRT activity (*17*), which is consistent with our observed maintenance of the clusters (fig. S6, C, D, and open circles in E). Collectively, the association and dissociation of the multienzyme complex reflected changes in cellular purine levels that were imposed by the addition of external reagents that regulate purine metabolic flux.

Azaserine – glutamine analogue – decreases purine biosynthesis

Basically, if the cell can't utilise the salvage pathway (due to lack on HGPRT), then it will increase the de novo pathway – will need to make more enzyme

Purine-depleted conditions

0 min 30 min -azaserine



Purine synthesis is carefully regulated

- Ribose-5-phosphate → PRRP: INHIBITED by AMP/GMP/IMP
- Glutamine-PRPP amidotransferase (reaction 1) is an allosteric enzyme:
  INHIBITED by AMP/ GMP/ IMP
  STIMULATED by PRPP
- Pathway is also controlled at two later steps involving IMP

  IMP dehydrogenase: INHIBITED by too much GMP / XMP

  Adenylosuccinate synthetase: INHIBITED by too much AMP

- NOTE: the roles of ATP and GTP in the pathway - If there is a shortage of ATP or GTP .... the steps in the pathway involving these molecules will not proceed. Also GTP promotes AMP production and ATP promotes GMP production

Ribose phosphate Pyrophosphokinase

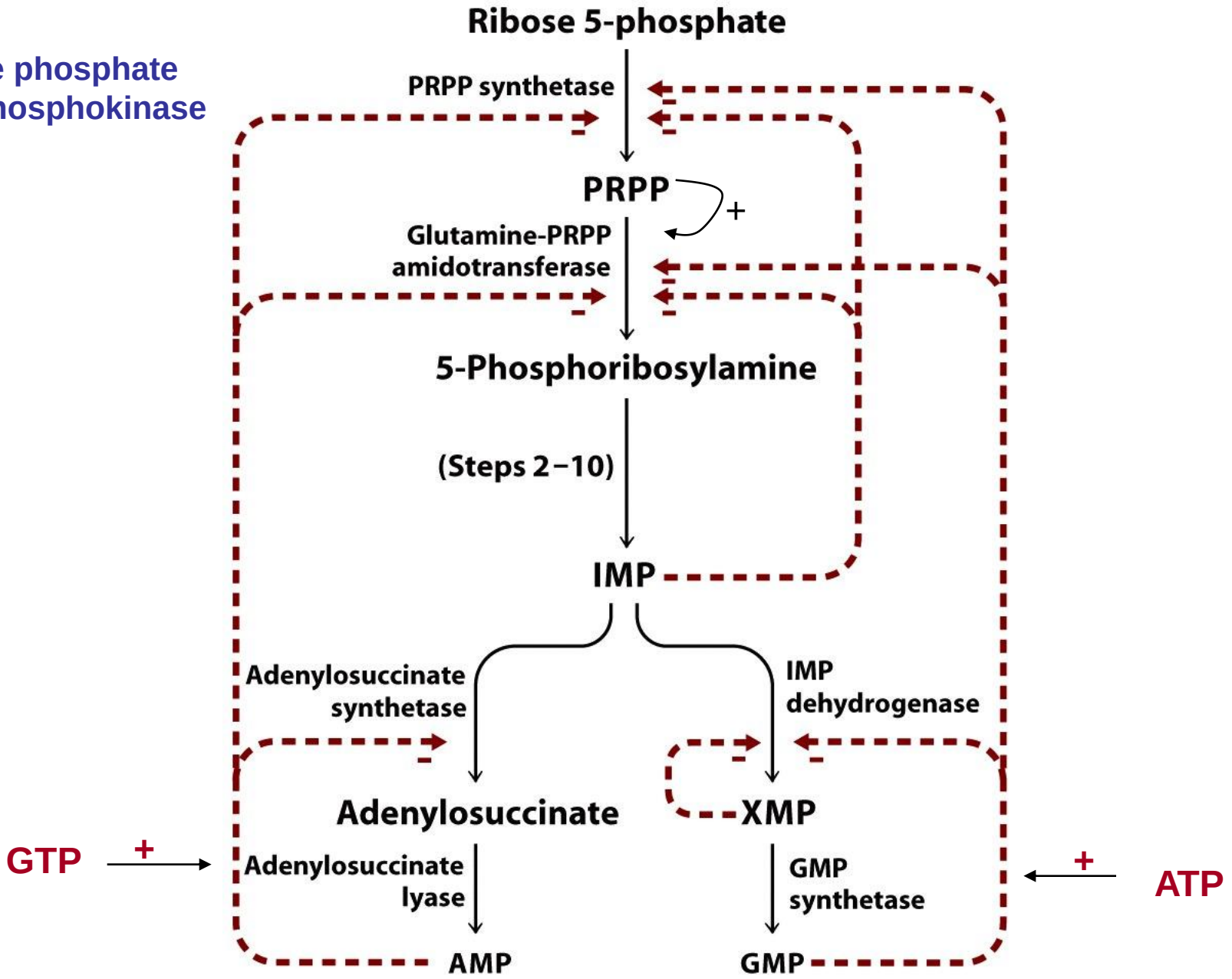


Figure 18-8 Principles of Biochemistry, 4/e