Inhibition of pyrimidine biosynthesis and salvage

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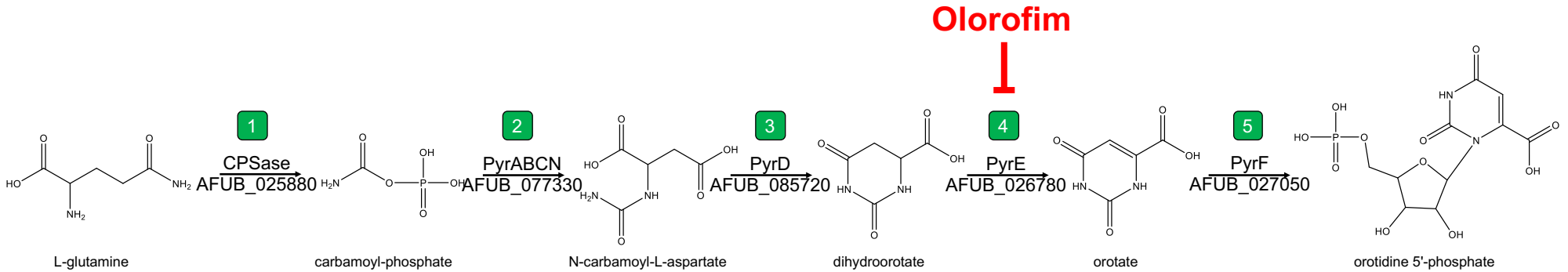
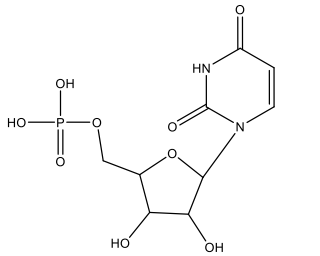
De novo pyrimidine synthesis

De novo synthesis of UMP – The 1st pyrimidine nucleotide



PyrG AFUB_024310 6



UMP

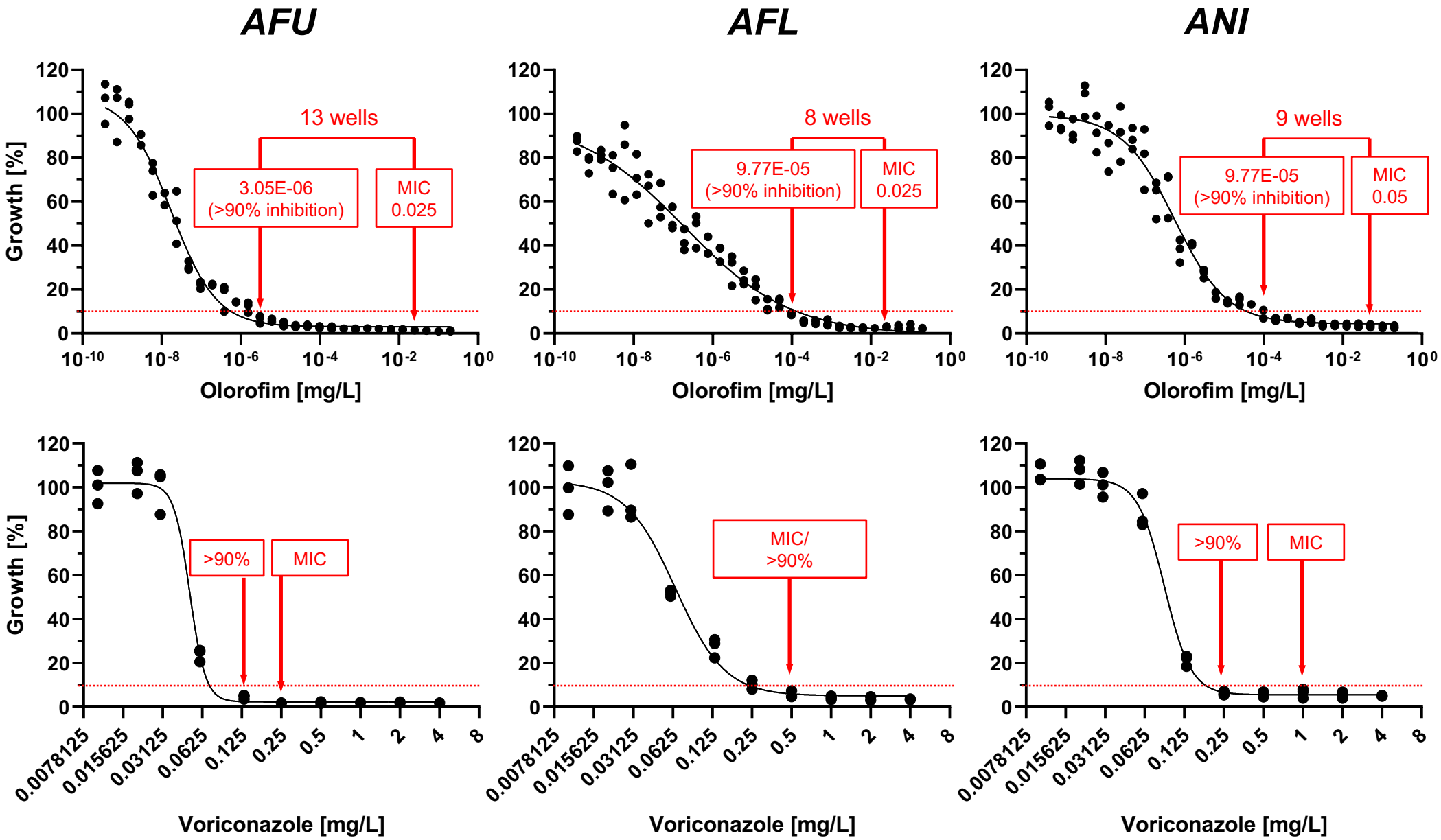
1. Carbamoyl-phosphate synthase (CPSase) AFUB_025880
2. Bifunctional pyrimidine biosynthesis protein (PyrABCN) AFUB_077330
3. Dihydroorotase (PyrD) AFUB_085720
4. Dihydroorotate reductase (PyrE) AFUB_026780
5. Orotate phosphoribosyltransferase (PyrF) AFUB_027050
6. Orotidine 5'-monophosphate decarboxylase (PyrG) AFUB_024310

de novo

salvage

1. Cytosine deaminase (FcyA) AFUB_005410
2. Uracil phosphoribosyltransferase (Uprt) AFUB_053020
3. Uridine dehydrogenase A (UdhA) AFUB_011230
4. Uridine kinase (Uk) AFUB_022460

Sub-MIC activity of olorofim on early stage-growth (12 h)



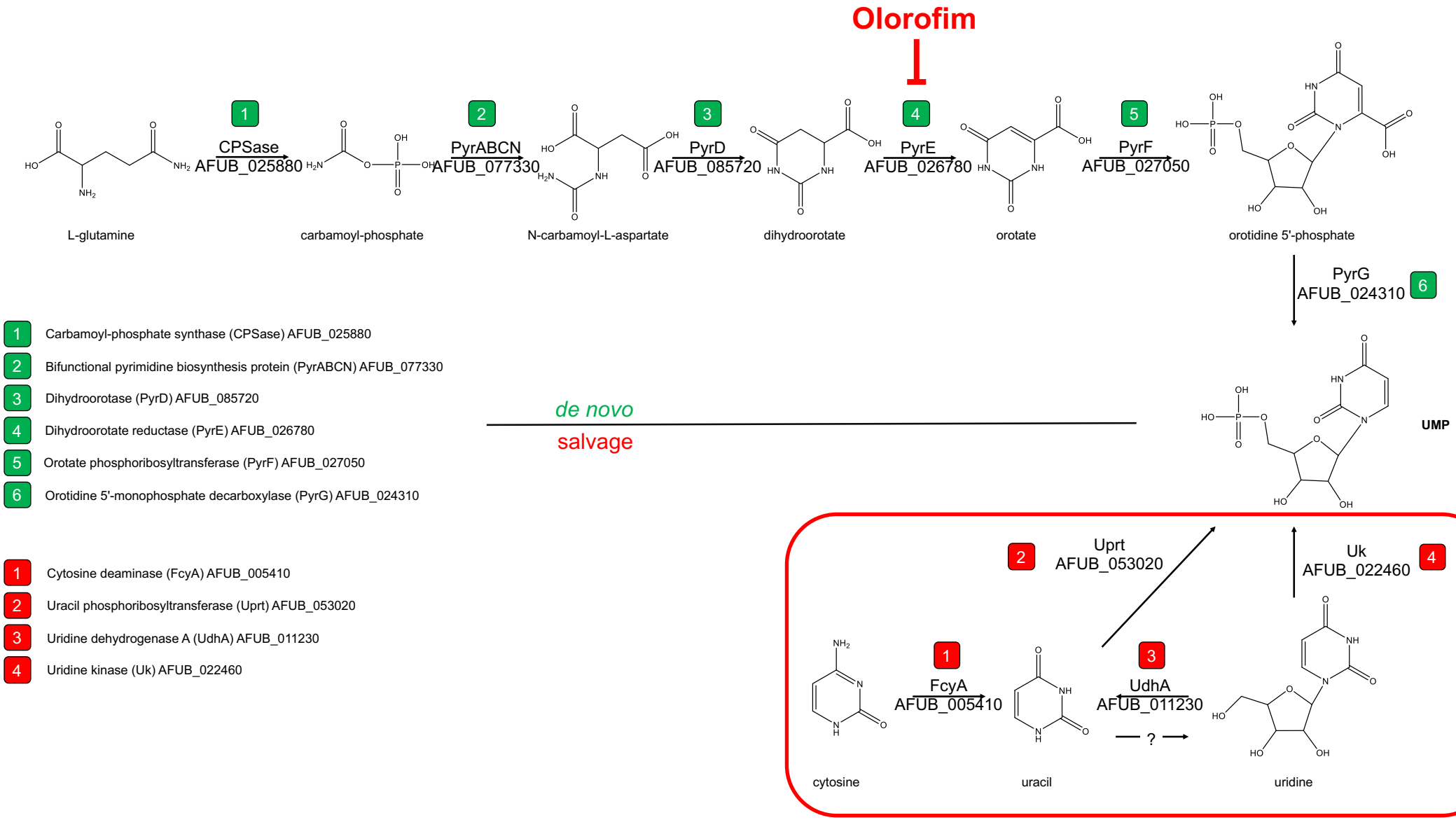
De novo synthesis of UMP – The 1st pyrimidine nucleotide

	Olorofim			Voriconazole			
	>90% inhibition	MIC(24 h)	MIC(48 h)	>90% inhibition	MIC(24 h)	MIC(48 h)	
AFU	$3.05*10^{-6}$	0.0001	0.025	0.125	0.125	0.25	[mg/L]
AFL	$9.77*10^{-5}$	0.0008	0.025	0.5	0.25	0.5	
ANI	$9.77*10^{-5}$	0.0063	0.050	0.25	0.5	1	
AFU	$6.12*10^{-6}$	0.0002	0.050	0.358	0.358	0.716	[µM]
AFL	$1.96*10^{-4}$	0.0016	0.050	1.431	0.716	1.431	
ANI	$1.96*10^{-4}$	0.0125	0.100	0.716	1.431	2.863	
AFU	58,467	1,827	14				Molar ratios voriconazole/ olorofim
AFL	7,308	457	29				
ANI	3,654	114	29				

Particularly during early-stage growth, very low levels of olorofim are required to significantly inhibit growth of main pathogenic *Aspergillus* species

The pyrimidine salvage pathway

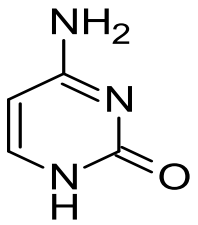
Synthesis of UMP – The pyrimidine salvage pathway



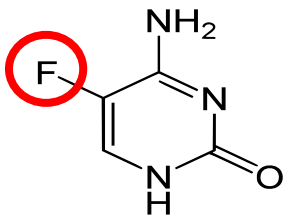
The antifungal 5-fluorocytosine

- nucleobase-analog antifungal drug class (only member)
- **<u>Prodrug: requires metabolic activation</u>**

Cytosine

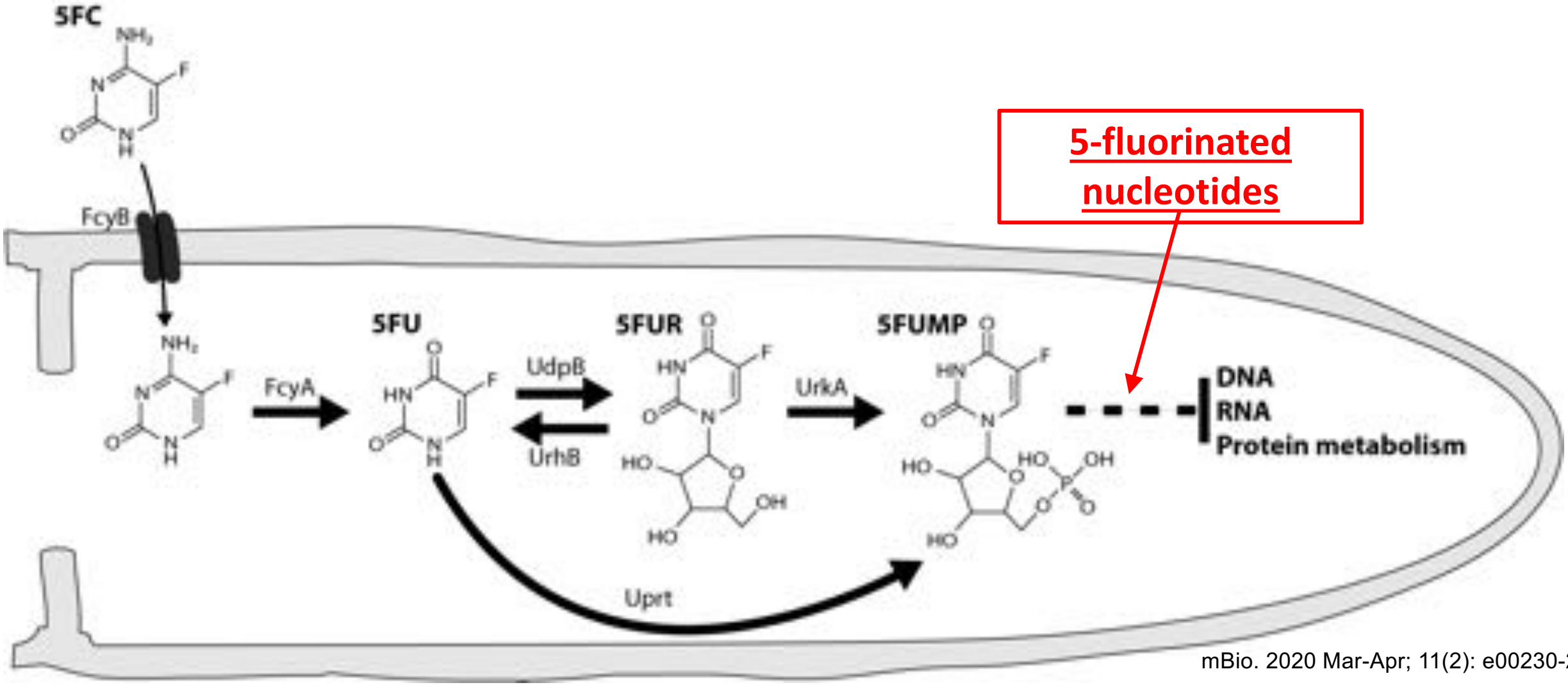


5FC



Metabolization of 5FC in *A. fumigatus*

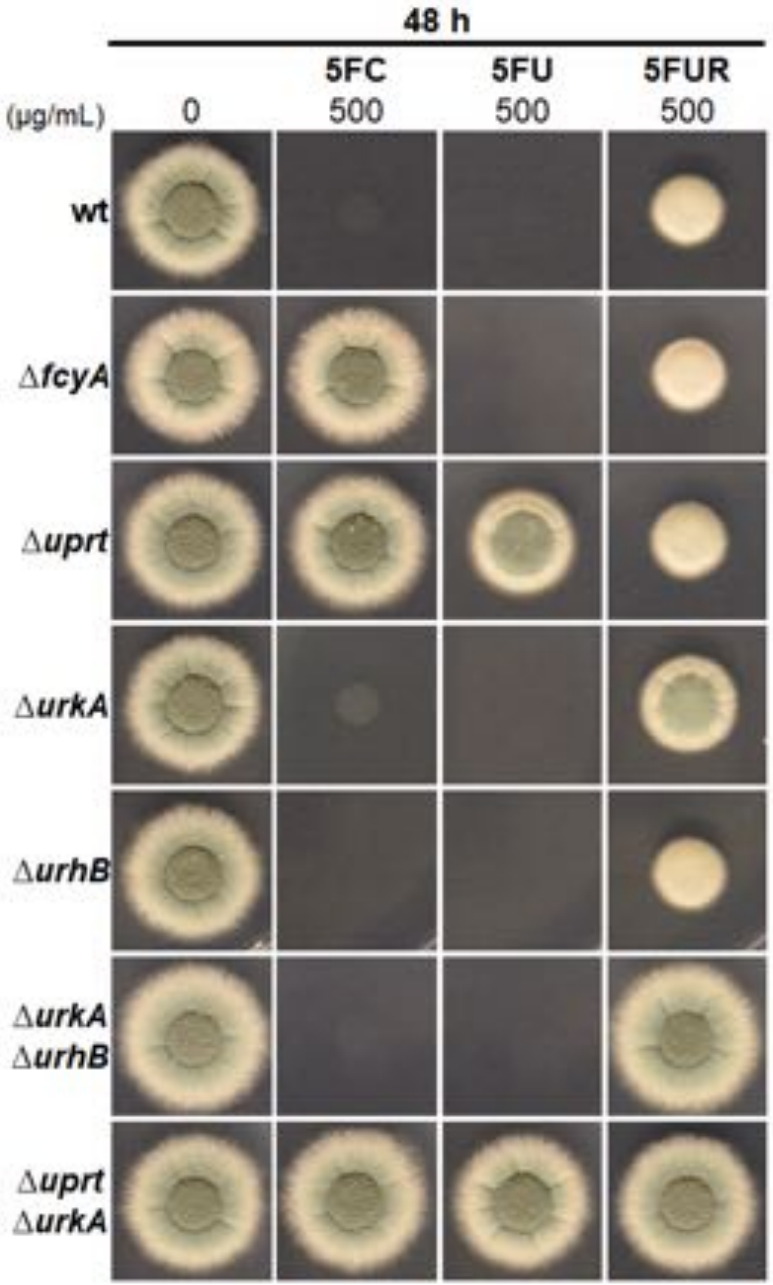
- **The classical route of metabolization occurs through FcyA and Uprt**
- **Additional enzymes UrhB, UdpB and UrkA are involved in the metabolization of 5FU and 5FUR**

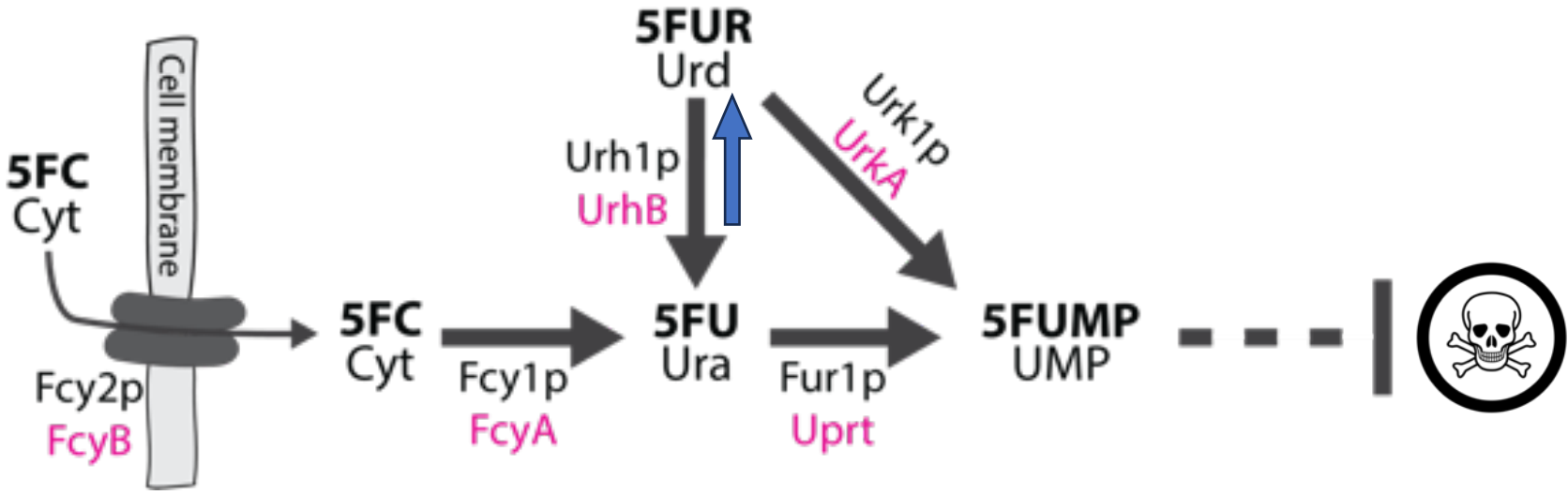


mBio. 2020 Mar-Apr; 11(2): e00230-20.

PLoS Pathog . 2022 Dec 27;18(12):e1011066.

Mutants showing the highest fluoropyrimidine resistance





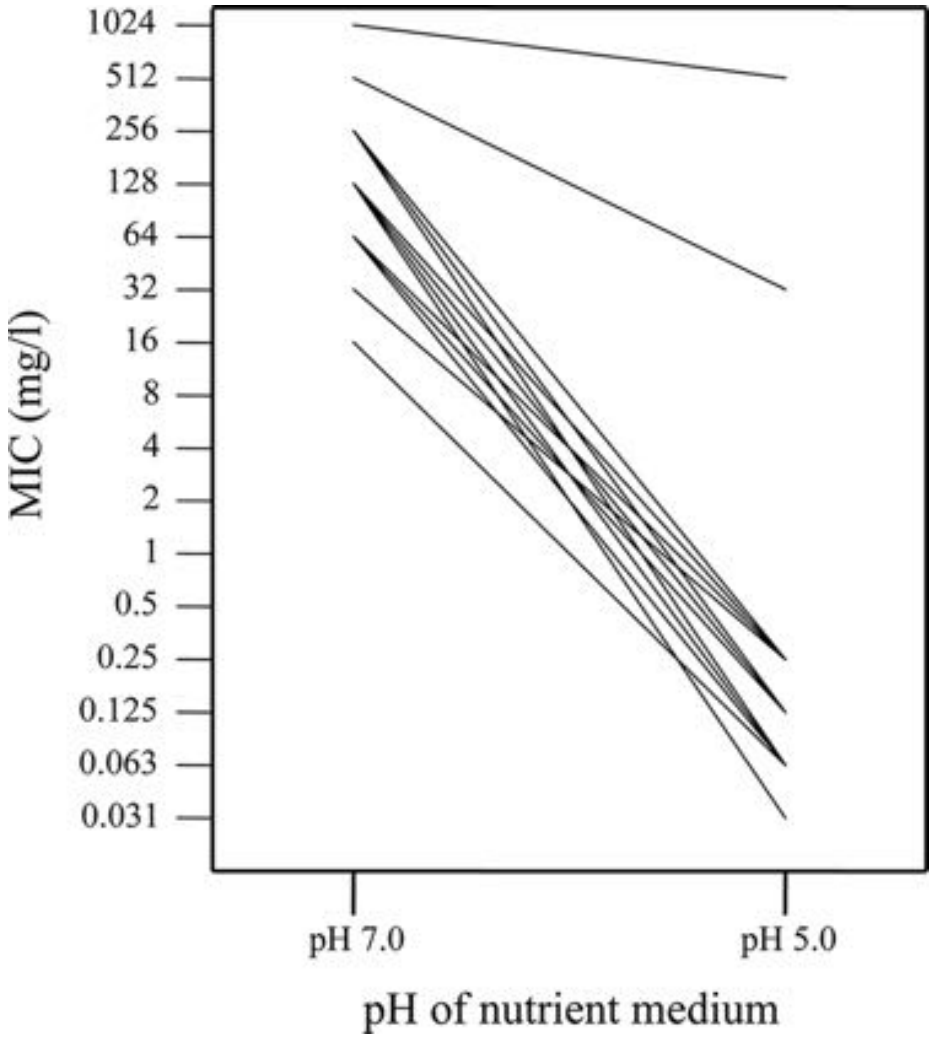
Black: ***S. cerevisiae*** **enzymes.**
Magenta: ***A. fumigatus*** **enzymes.**

Clinical use of 5FC for aspergillosis

- ***Rarely used for the treatment aspergillosis***

What's the problem with 5FC against A. fumigatus?

5FC activity is highly pH dependent



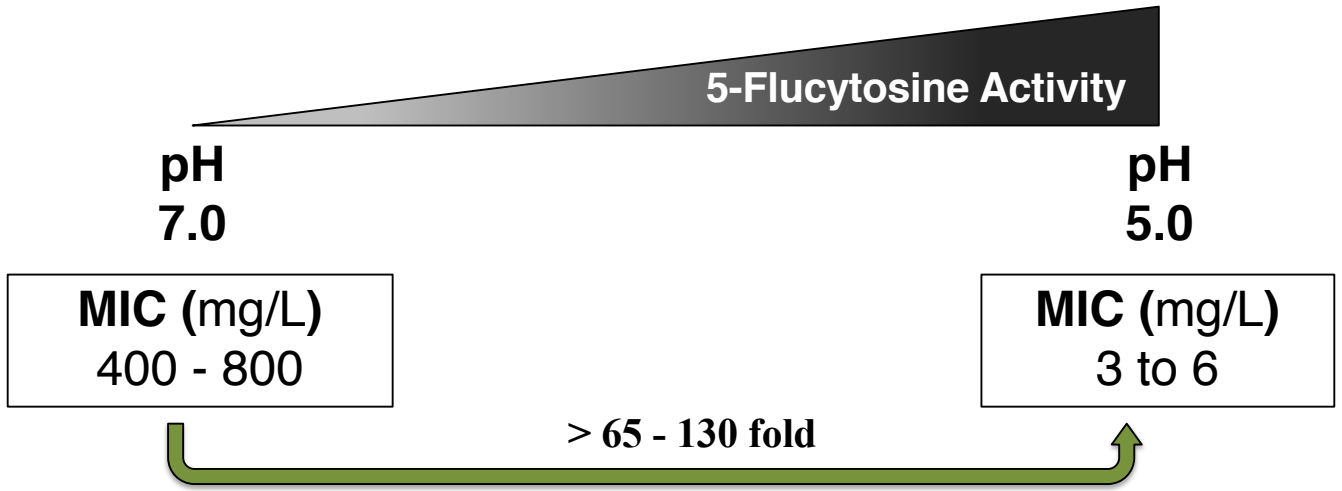
pH 7.0 / pH 5.0 correlation

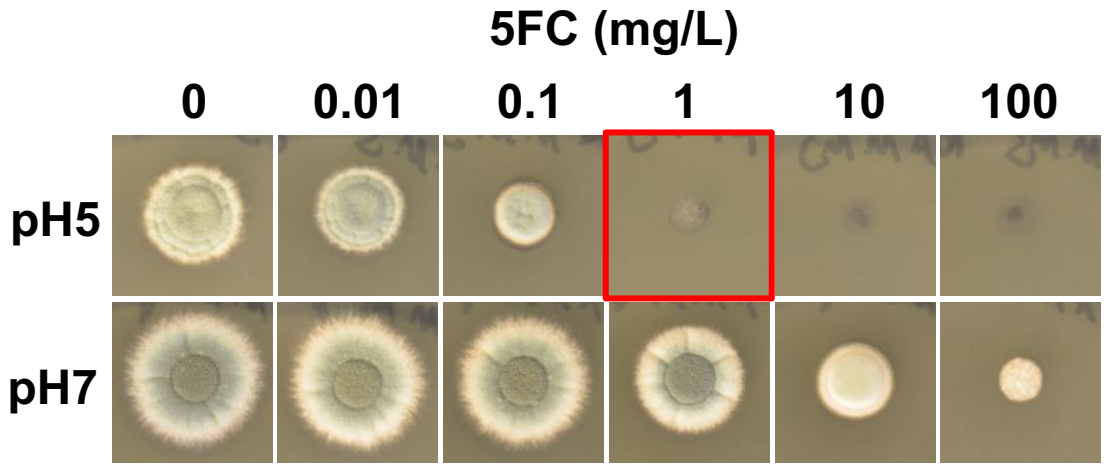
- **insignificant activity at neutral pH, very potent at low pH**

Antimicrob Agents Chemother. 2004 Aug; 48(8): 3147–3150.

Antimicrob Agents Chemother. 2008 Dec;52(12):4483-5.

A. fumigatus intrinsic 5FC resistance



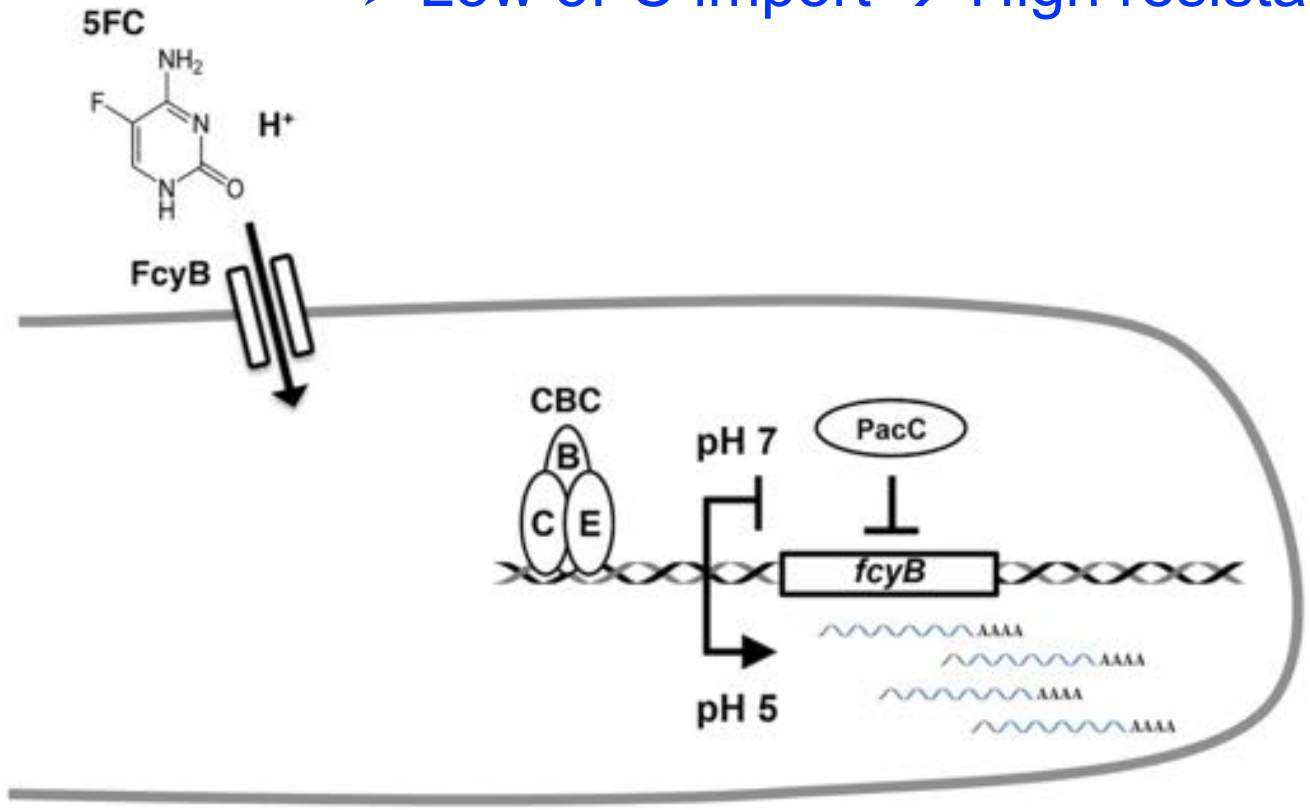


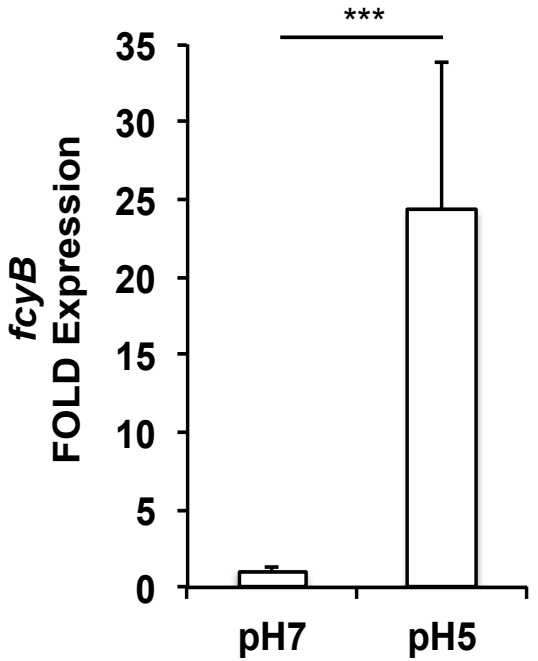
Antimicrob Agents Chemother. 2018 Jun; 62(6): e02593-17.

A. fumigatus intrinsic 5FC resistance

The CBC and PacC repress *fcyB* expression at neutral pH

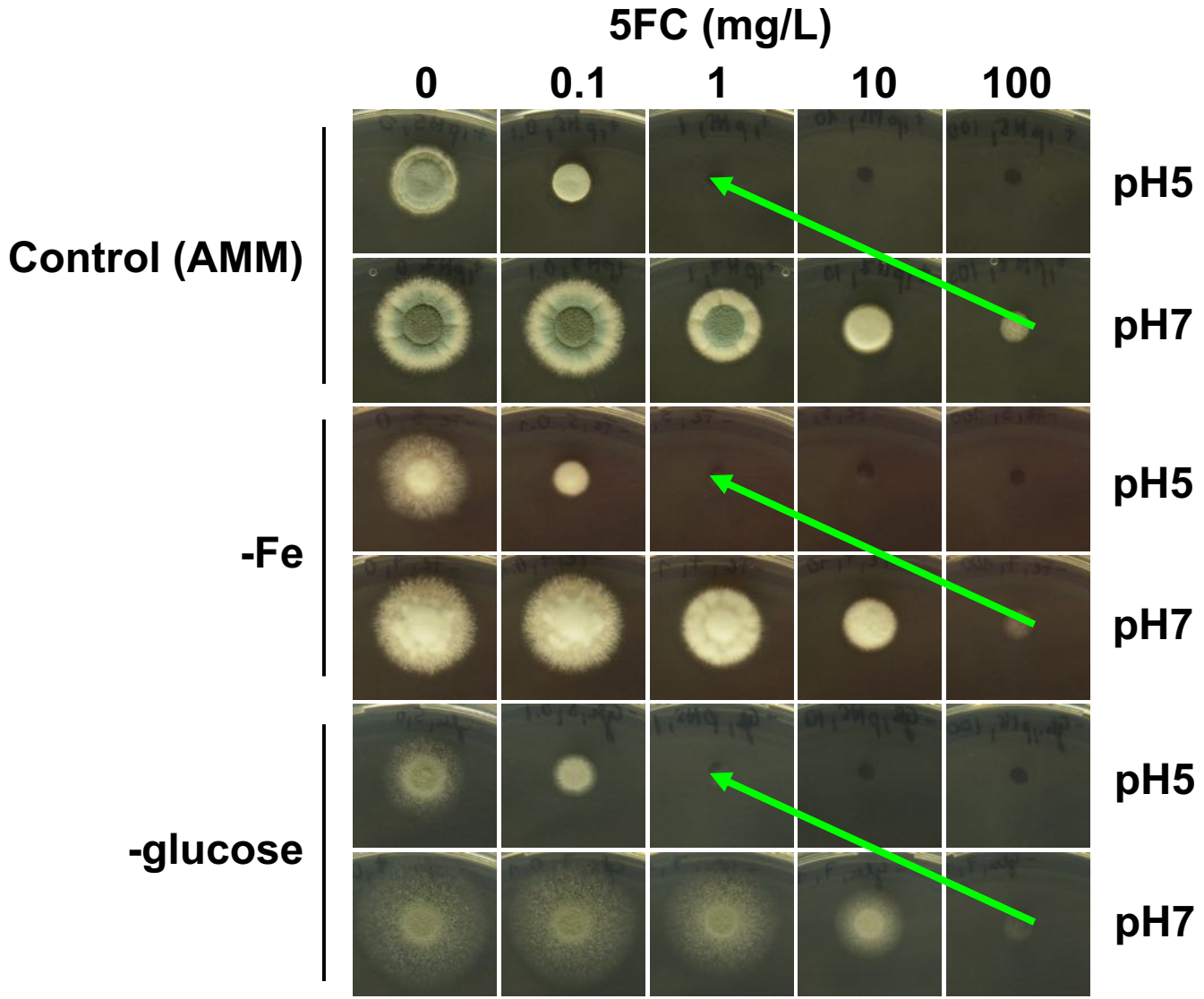
- Low 5FC import → High resistance



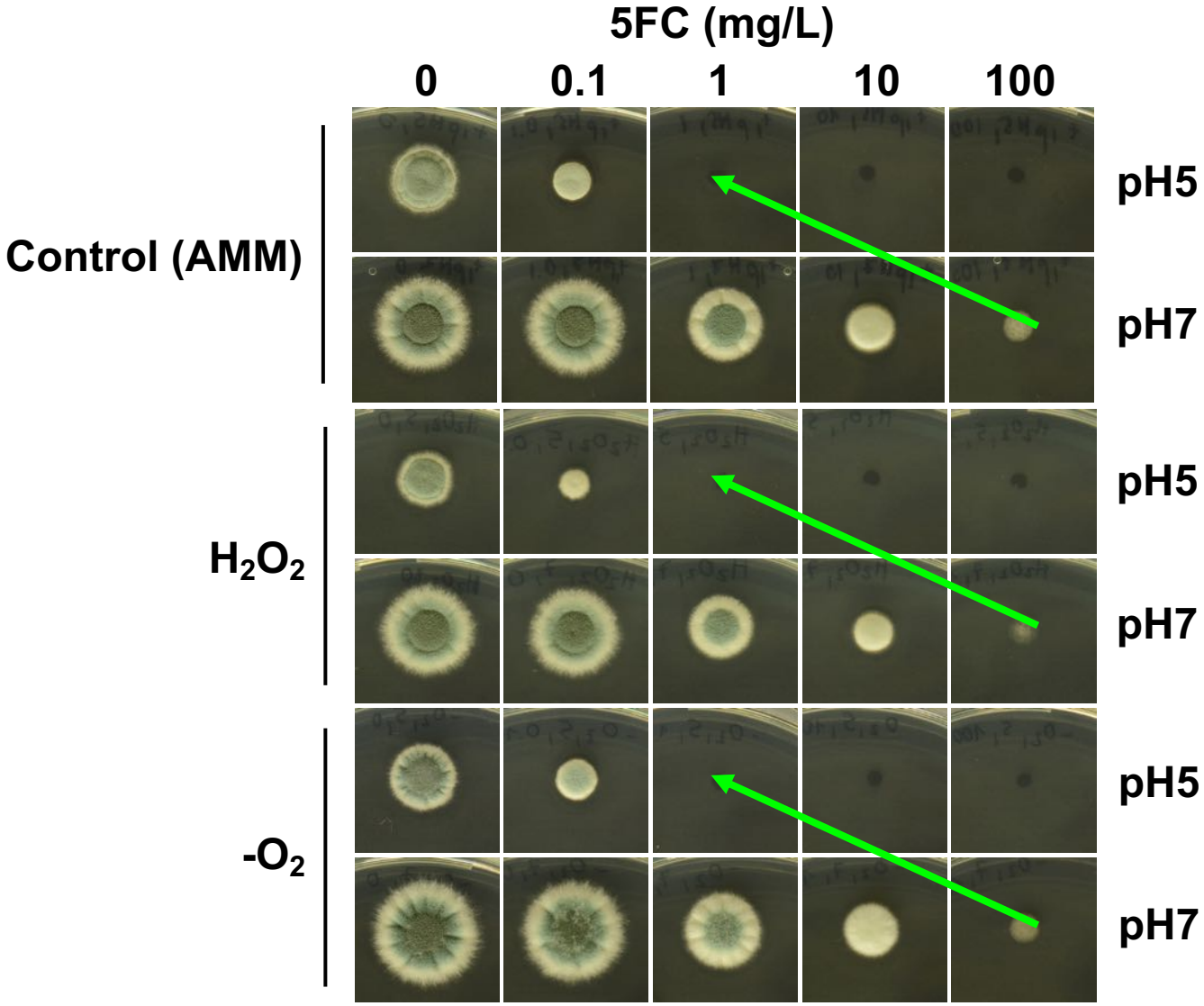


Antimicrob Agents Chemother. 2018 Jun; 62(6): e02593-17.

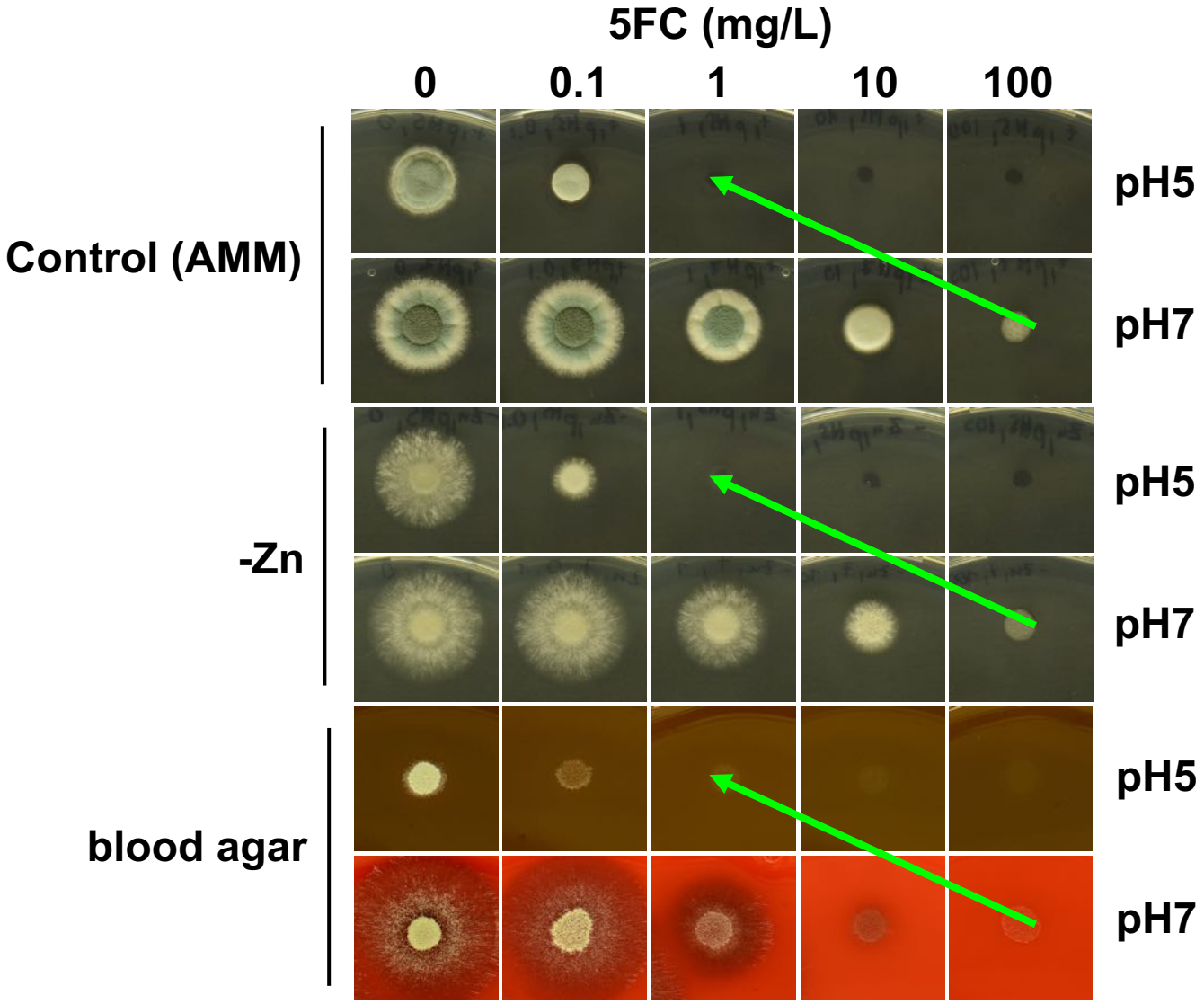
Environmental 5FC activity determinants



Environmental 5FC activity determinants

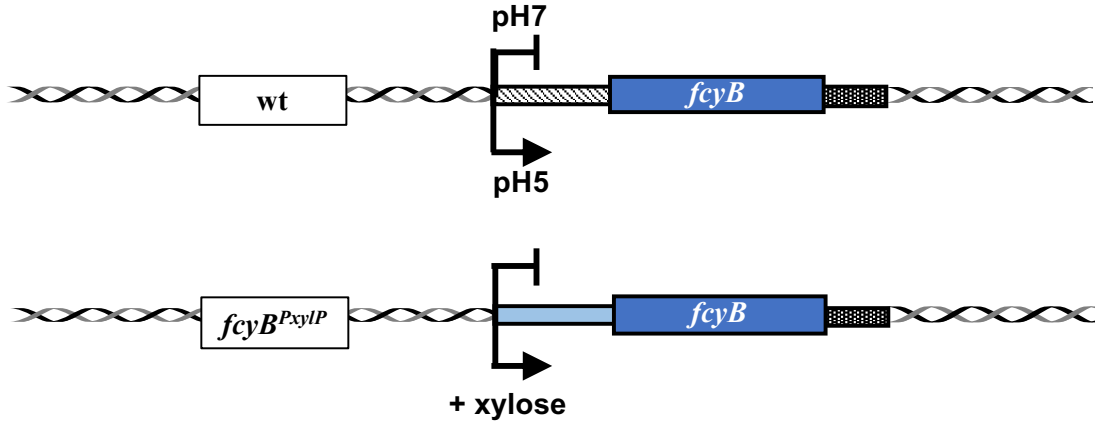


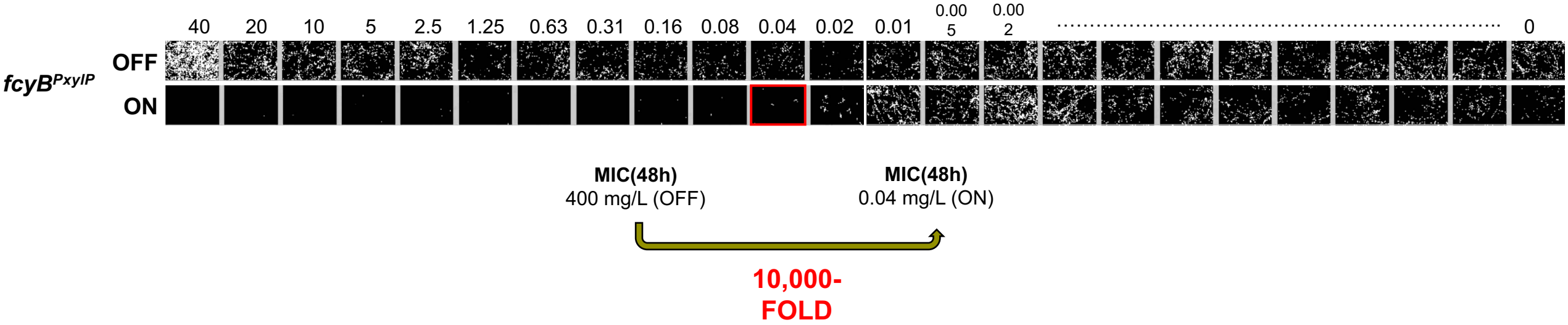
Environmental 5FC activity determinants



pH seems the main activity determinant

Bypassing transcriptional repression of *fcyB*





MIC(48h)
400 mg/L (OFF)

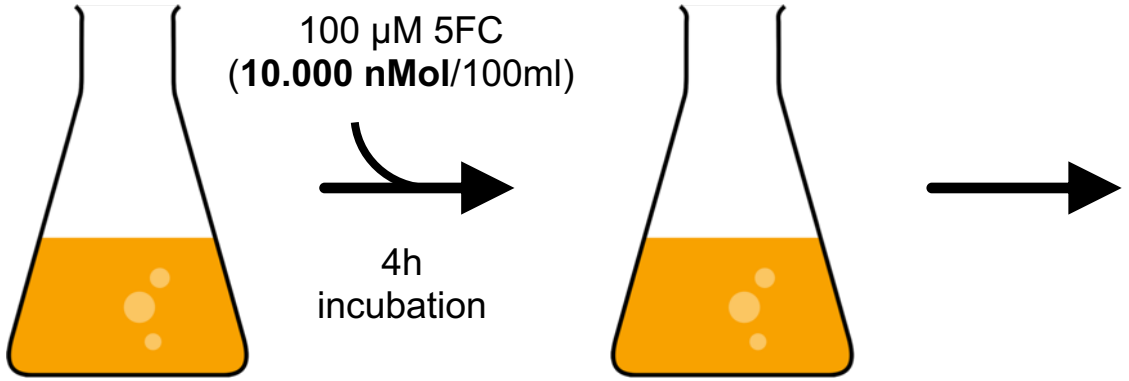
MIC(48h)
0.04 mg/L (ON)

10,000-FOLD

Reduced uptake is a major problem

Is high pH the only problem that mitigates 5FC activity against A. fumigatus?

Efflux of fluoropyrimidines during 5FC treatment



wt and mutants with fluoropyrimidine resistance phenotypes

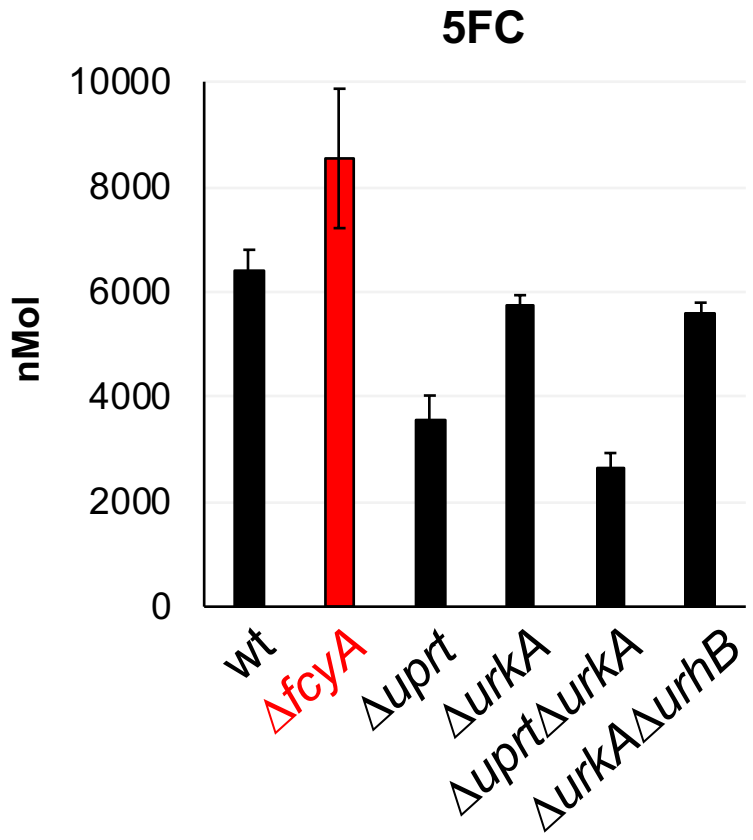
Quantification
5FC, 5FU, 5FUR

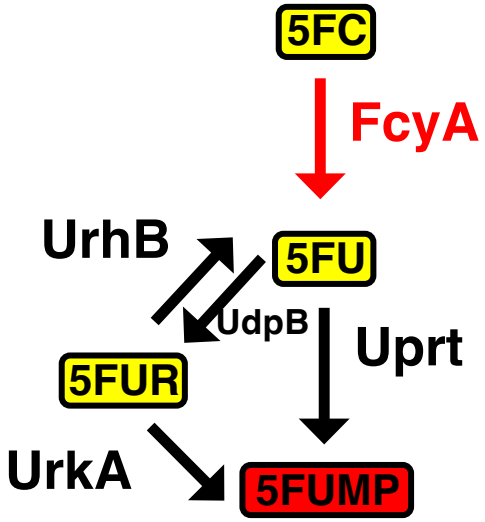
supernatant

efflux

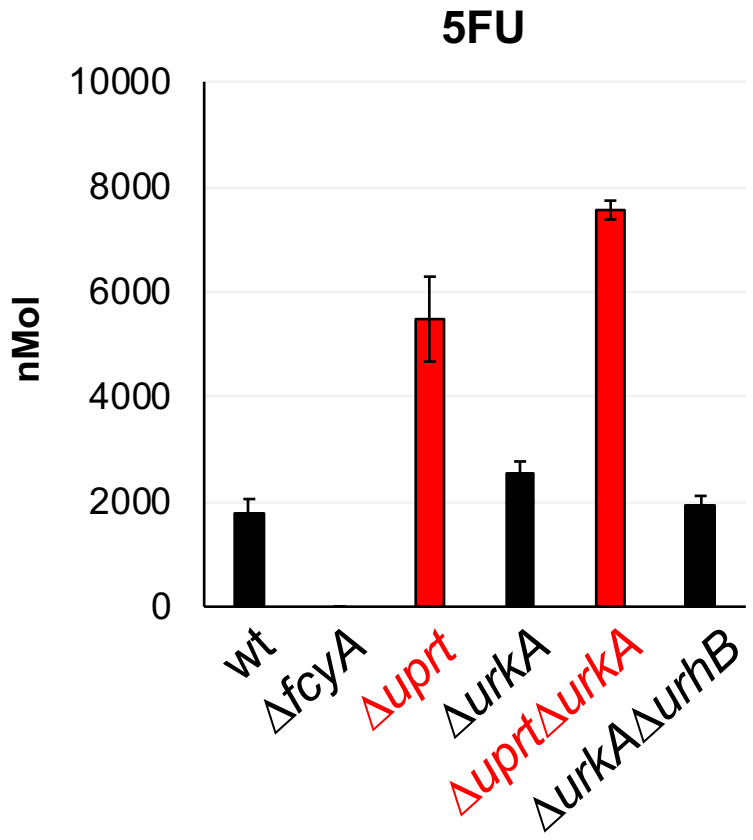
Franz Bracher
Christoph Müller
Anna Niedrig

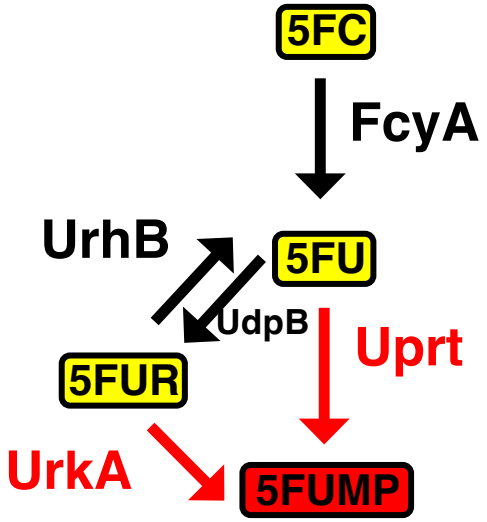
Extracellular 5FC levels



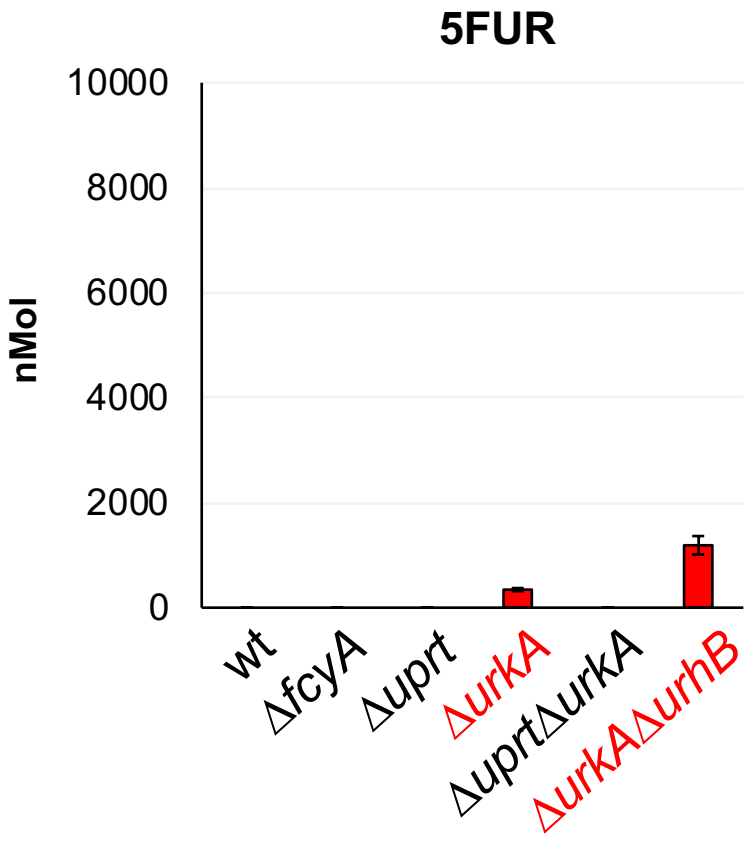


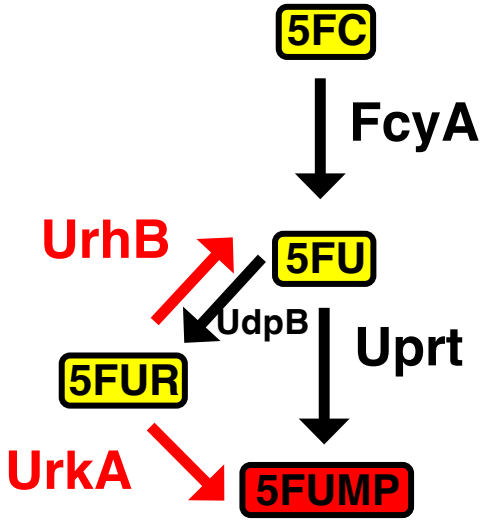
Extracellular 5FU levels





Extracellular 5FUR levels

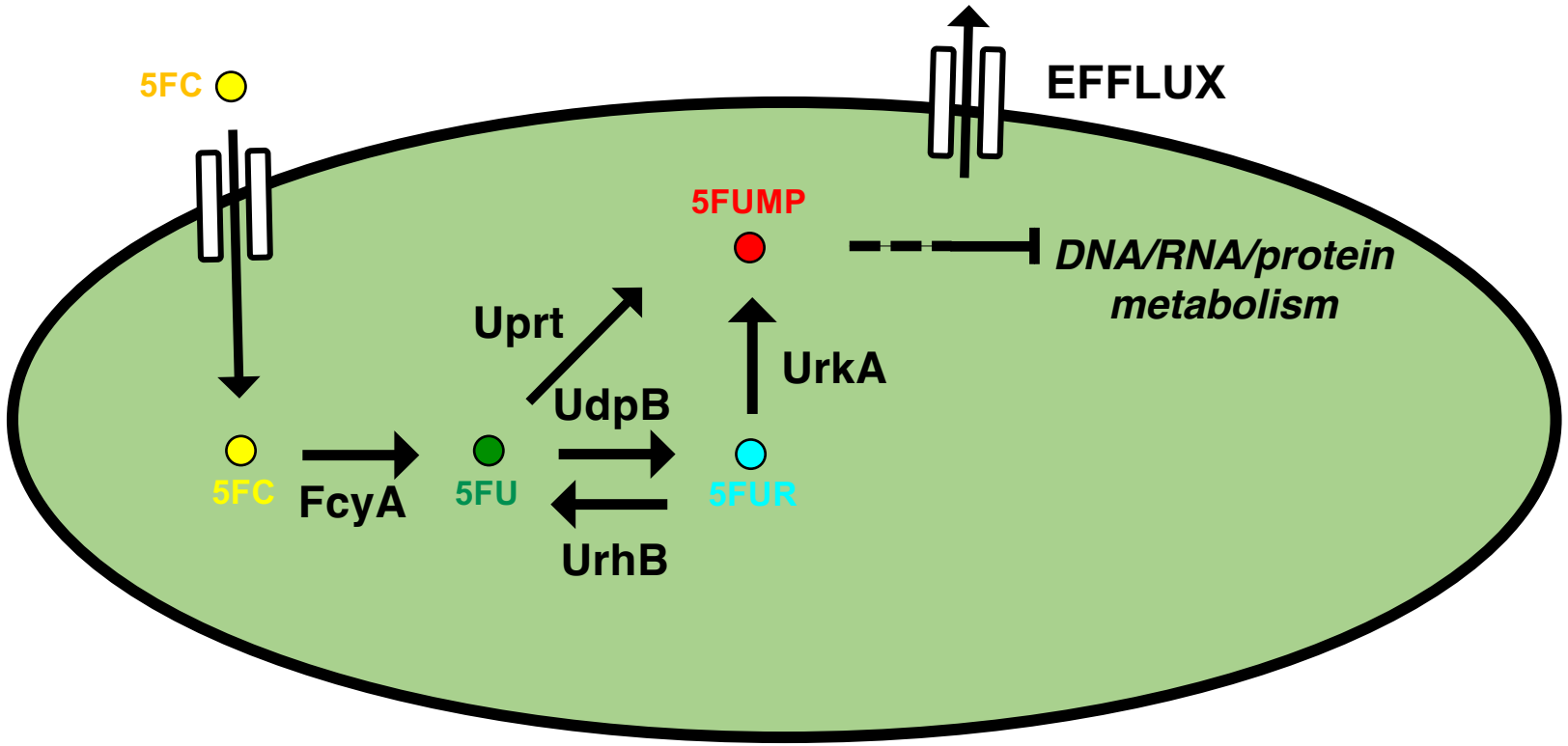




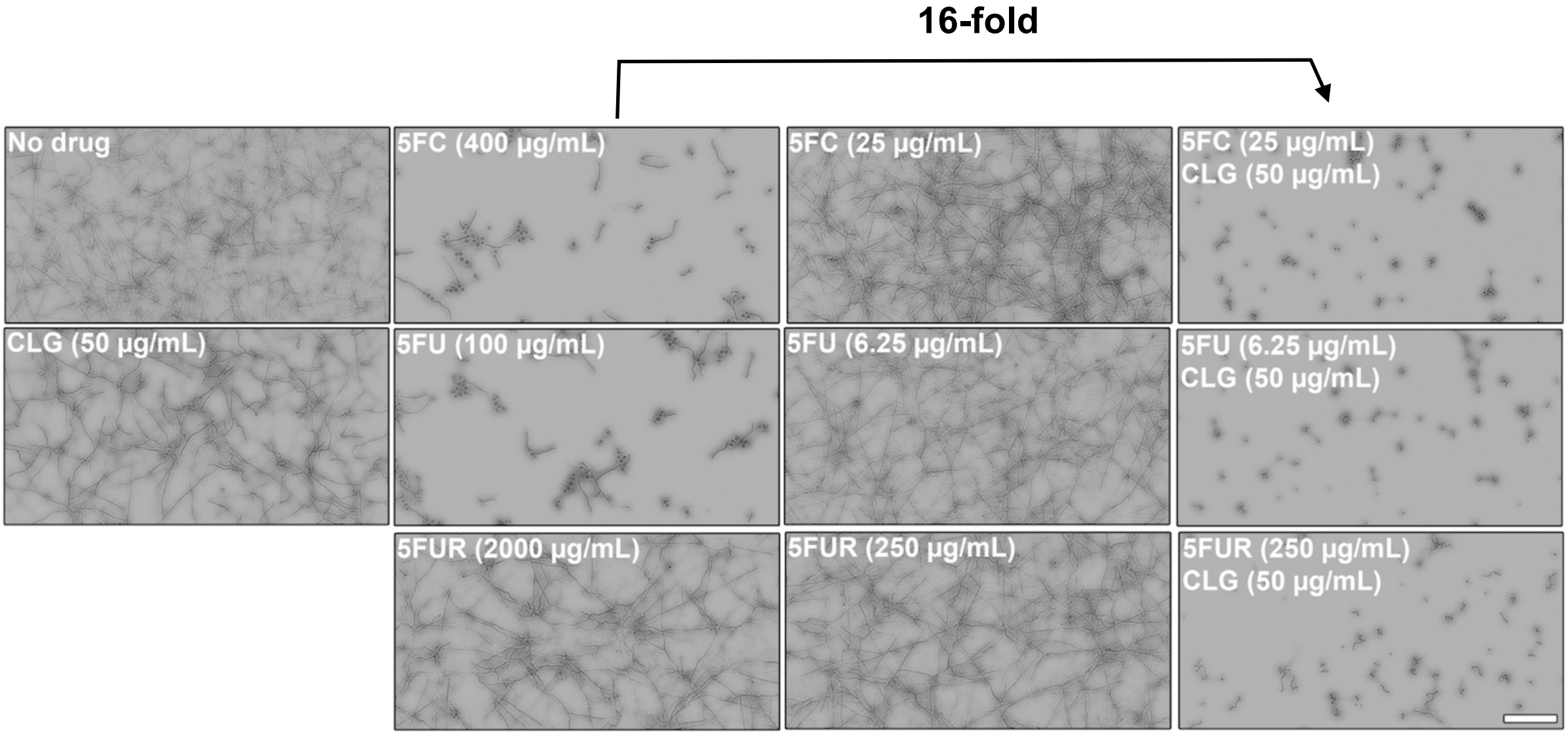
Fluoropyrimidine efflux during 5FC treatment

During 5FC exposure, *A. fumigatus* cells export 5FC-derived fluoropyrimidines into the environment

- increased resistance



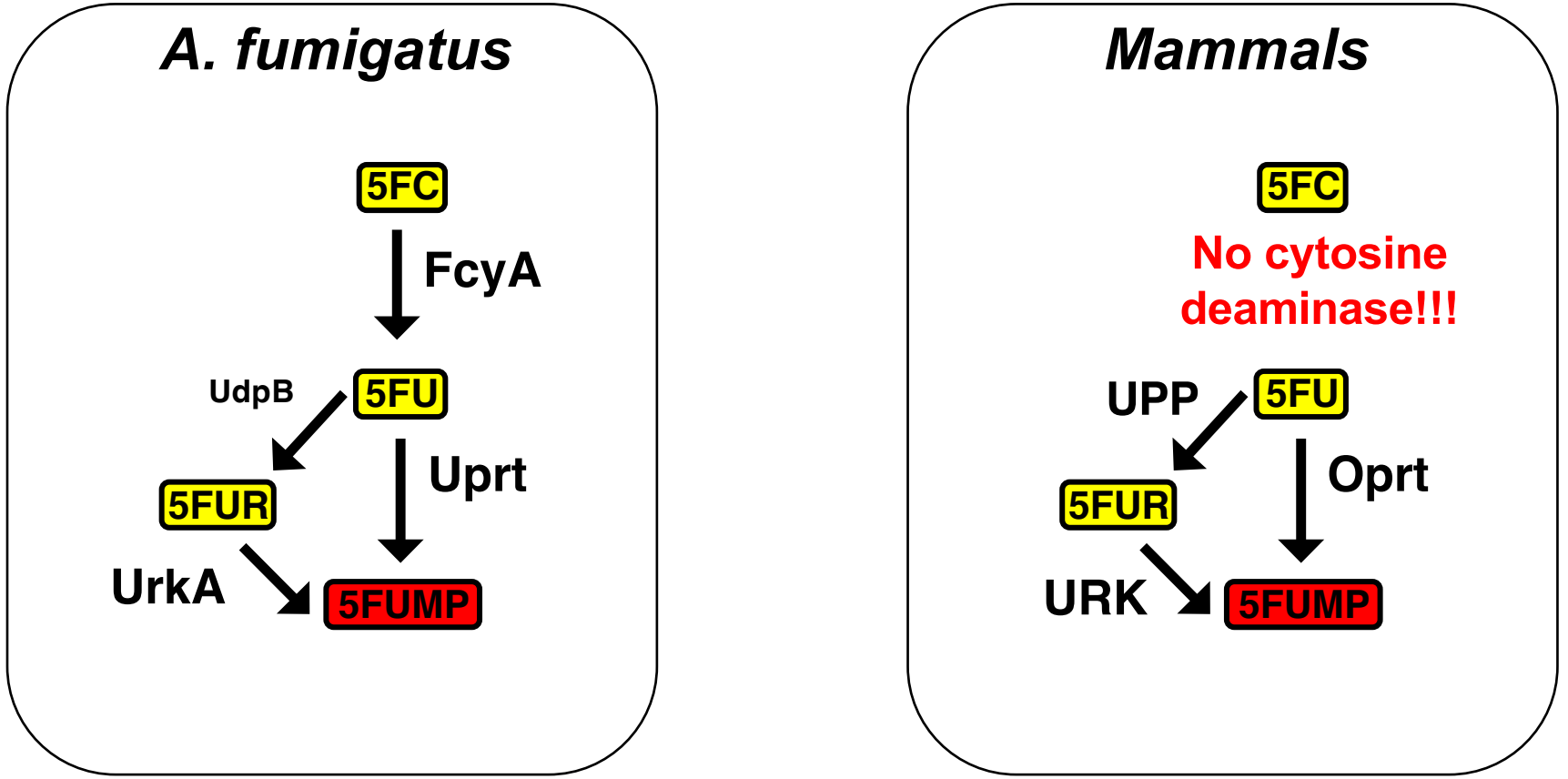
Interaction of 5FC, 5FU and 5FUR with the efflux inhibitor Clorgyline



CLG acts synergistic with 5FC and its derivatives

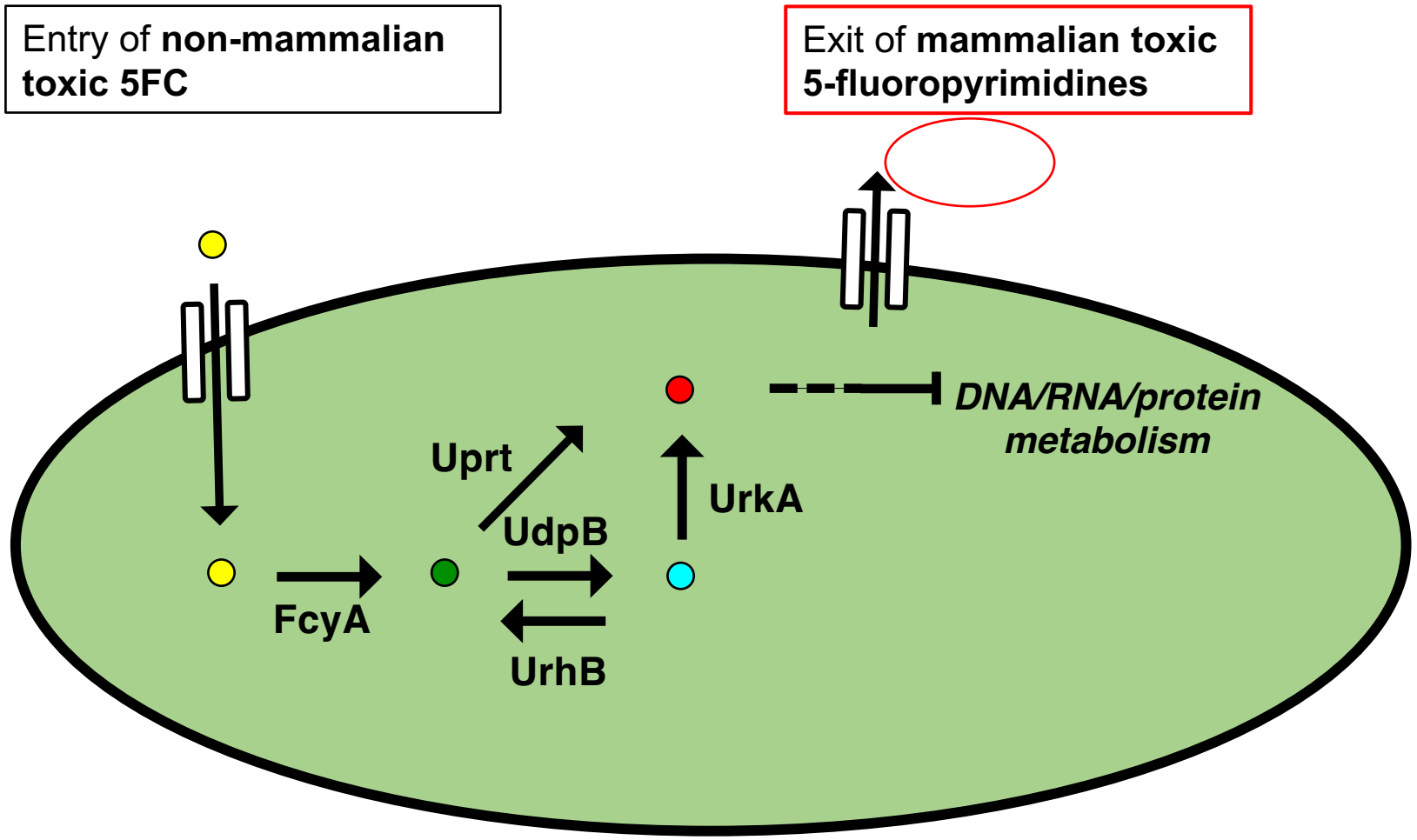
Could extruded fluoropyrimidines have adverse effect on host cells?

Fungal and mammalian pyrimidine salvaging



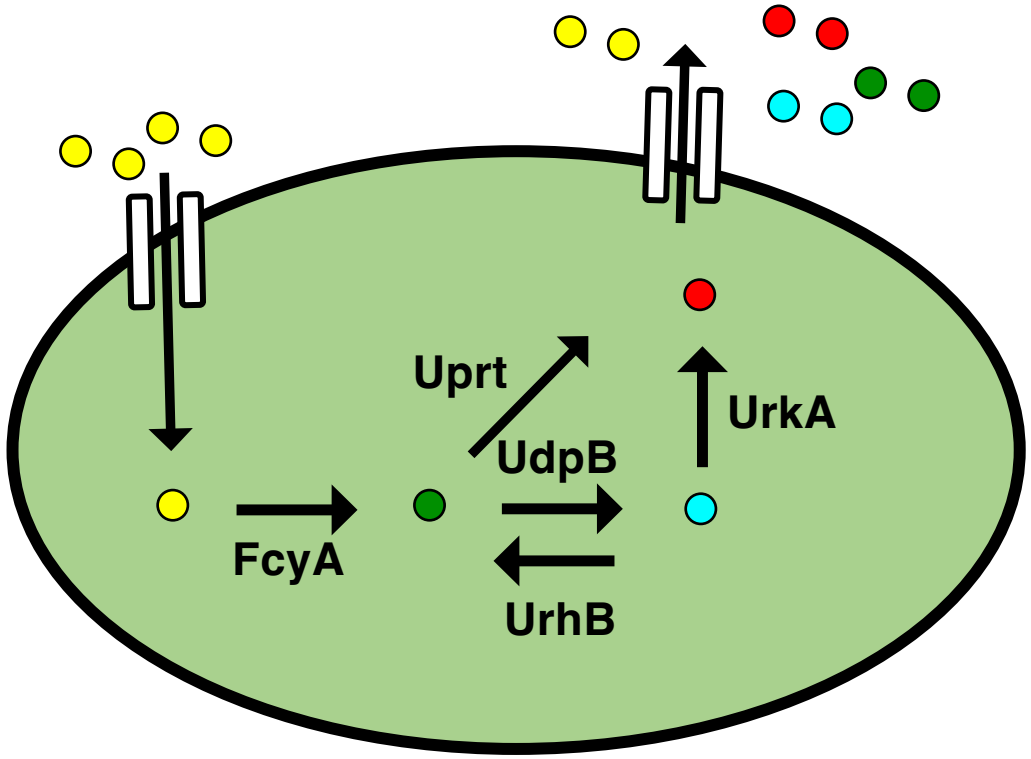
Resembles an *fcyA* null mutant

Efflux of fluoropyrimidines during 5FC treatment

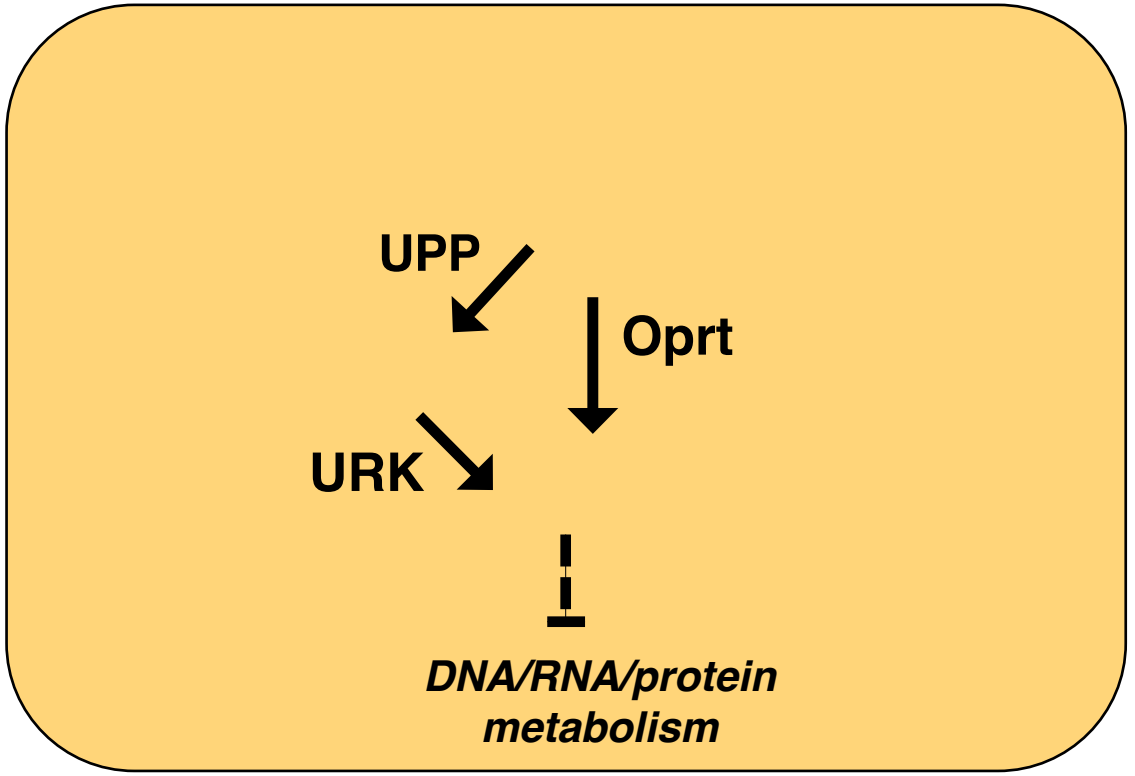


Fungal and mammalian pyrimidine salvaging

FUNGAL CELL
exposed to 5FC

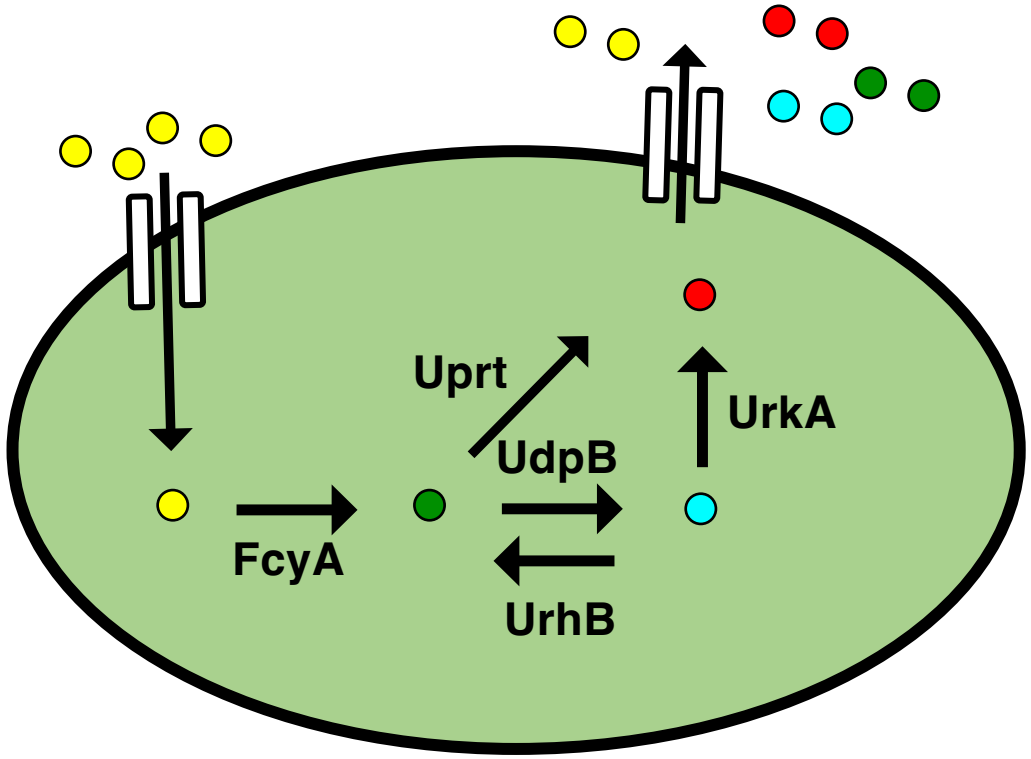


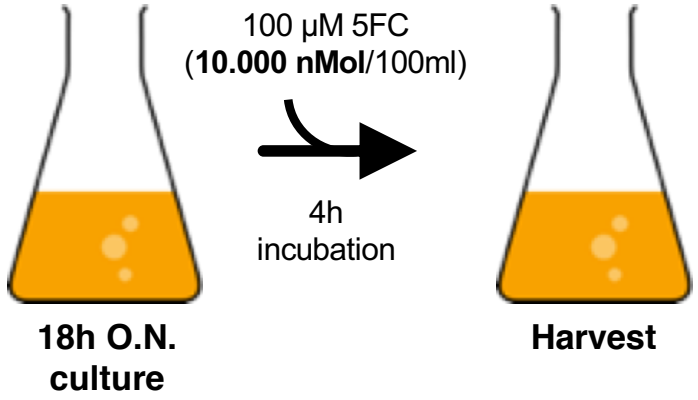
HOST CELL



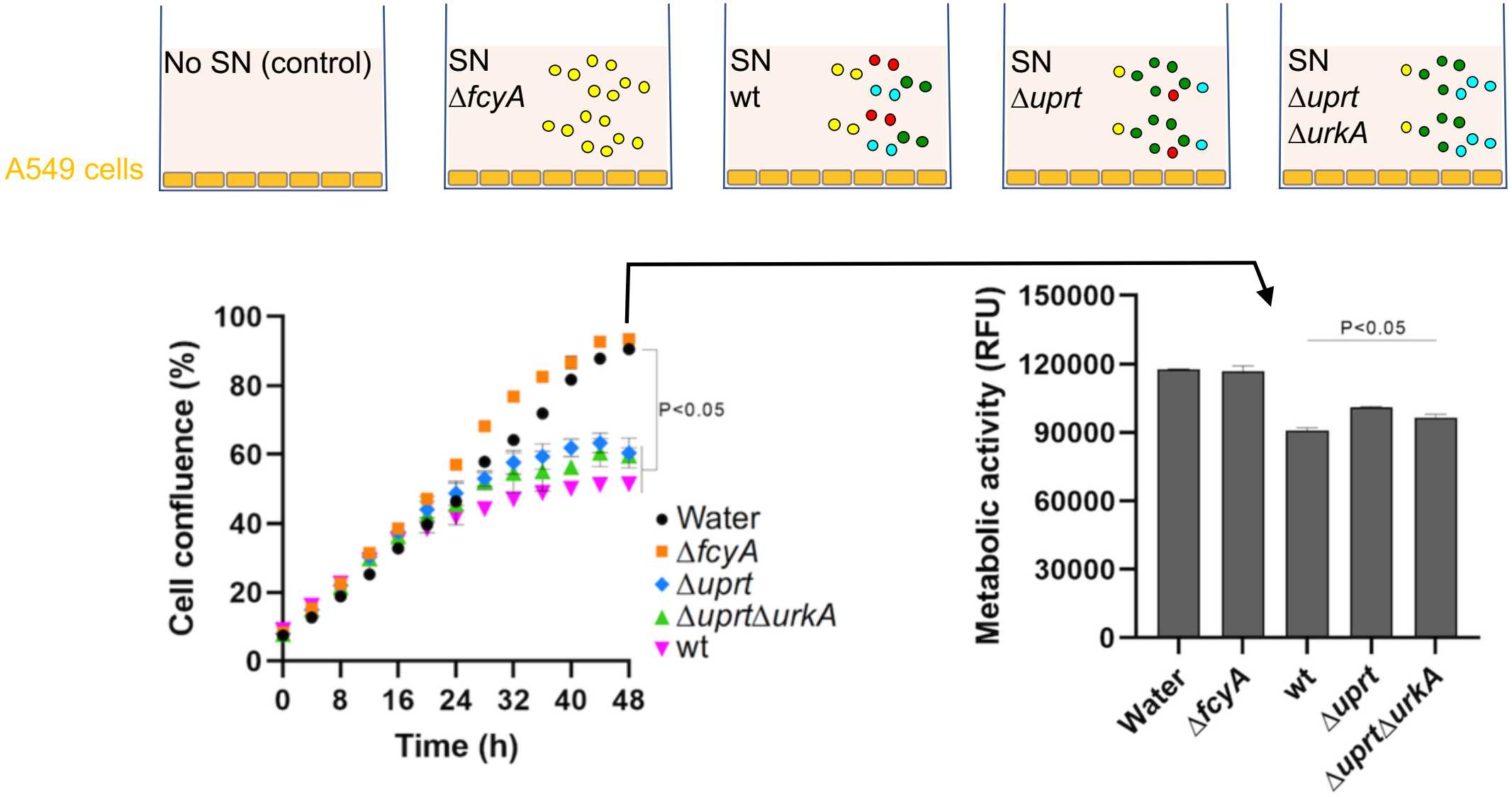
Predicted fluoropyrimidines extruded by different strains

Supernatants





Effects of effluxed fluoropyrimidines on mammalian cells



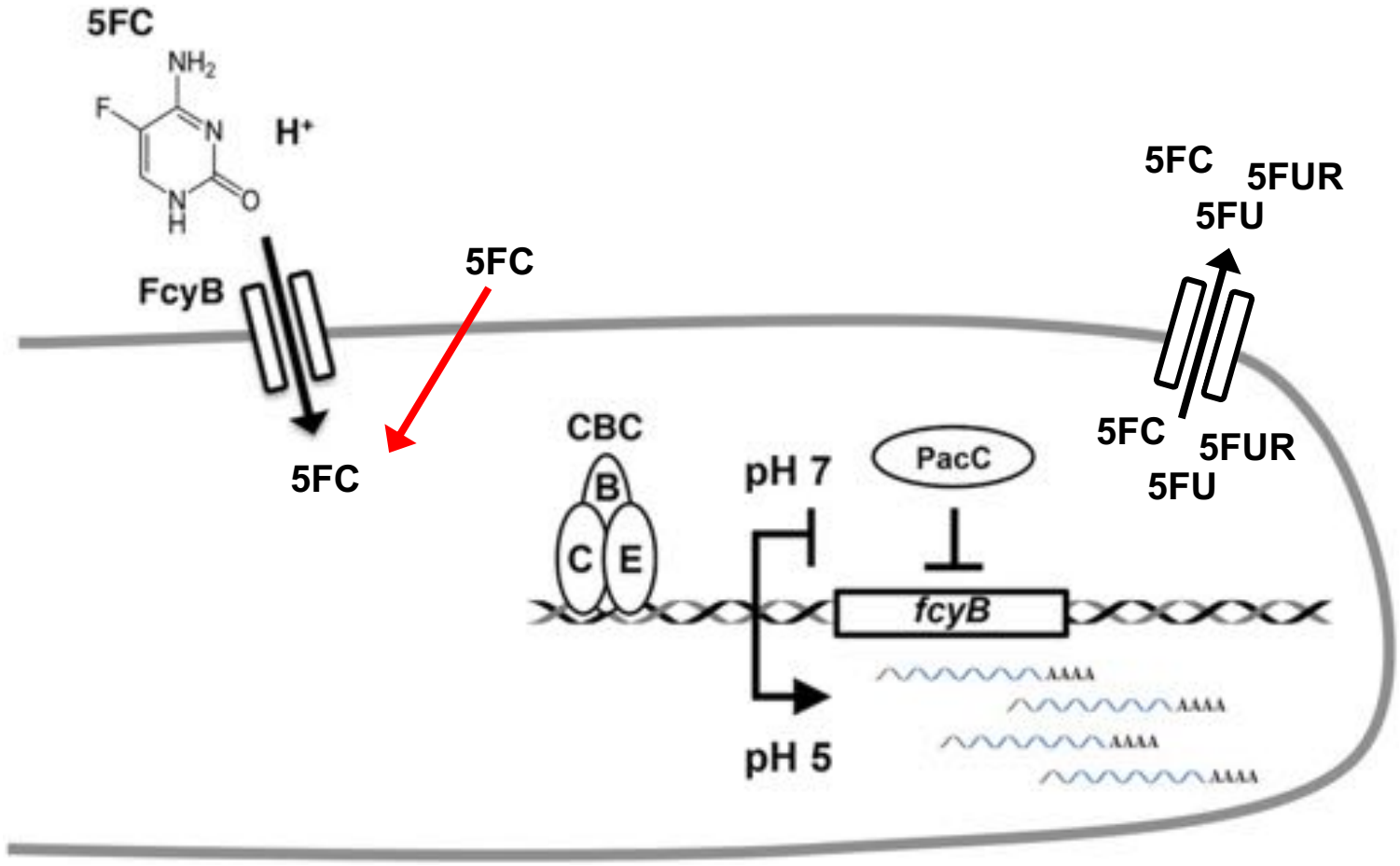
Effluxed 5FC derived fluoropyrimidines exert adverse effects on host cells.

Conclusions

Inhibit CBC, PacC or upstream cascades

Reduce/Inhibit efflux of 5FC and its derivatives

Develop strategies that promote 5FC delivery into the cell



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FWF Der Wissenschaftsfonds.