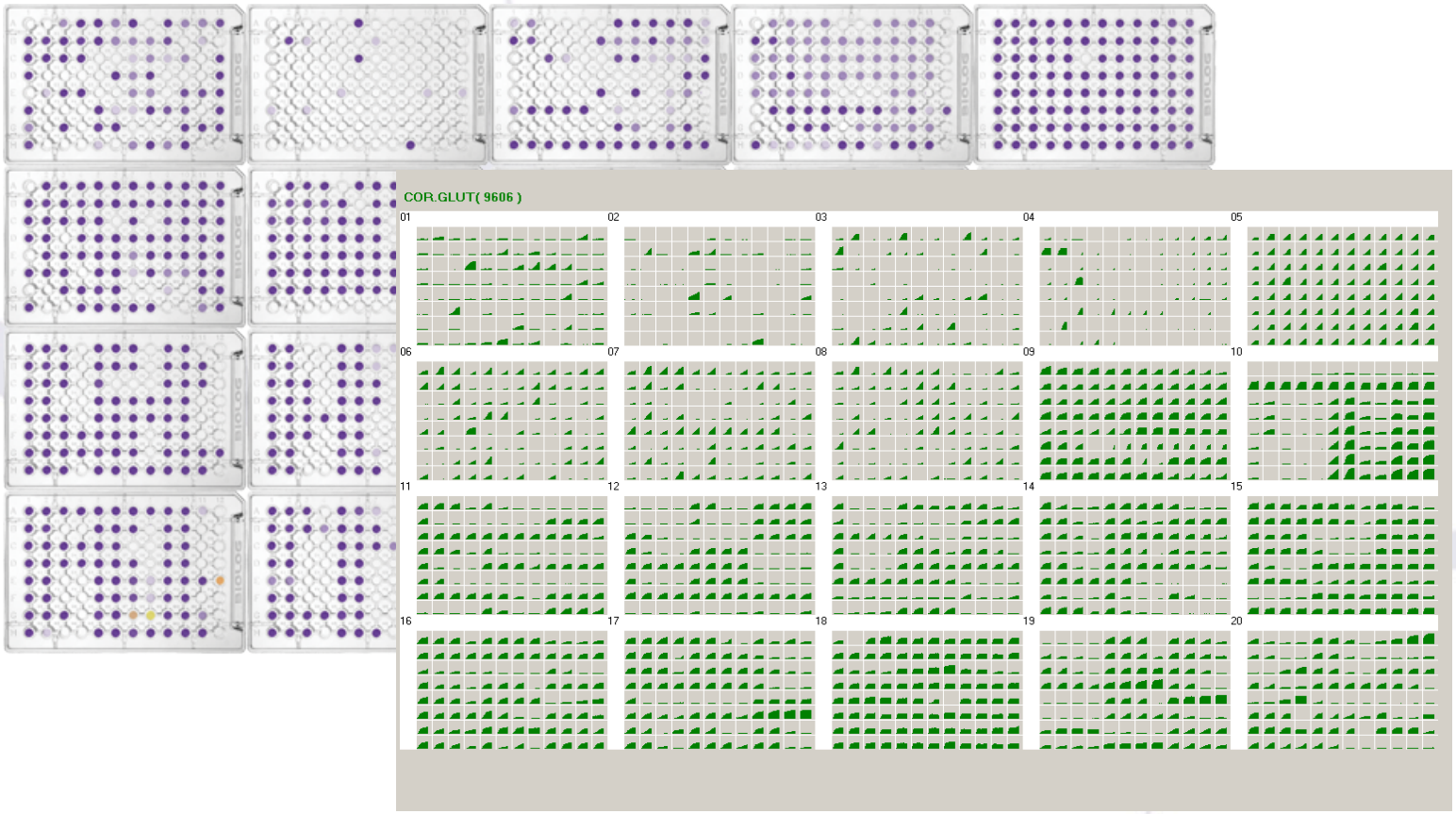
Conference on Predicting Cell Metabolism and Phenotypes



Barry Bochner, Biolog, Inc.,
bbochner@biolog.com

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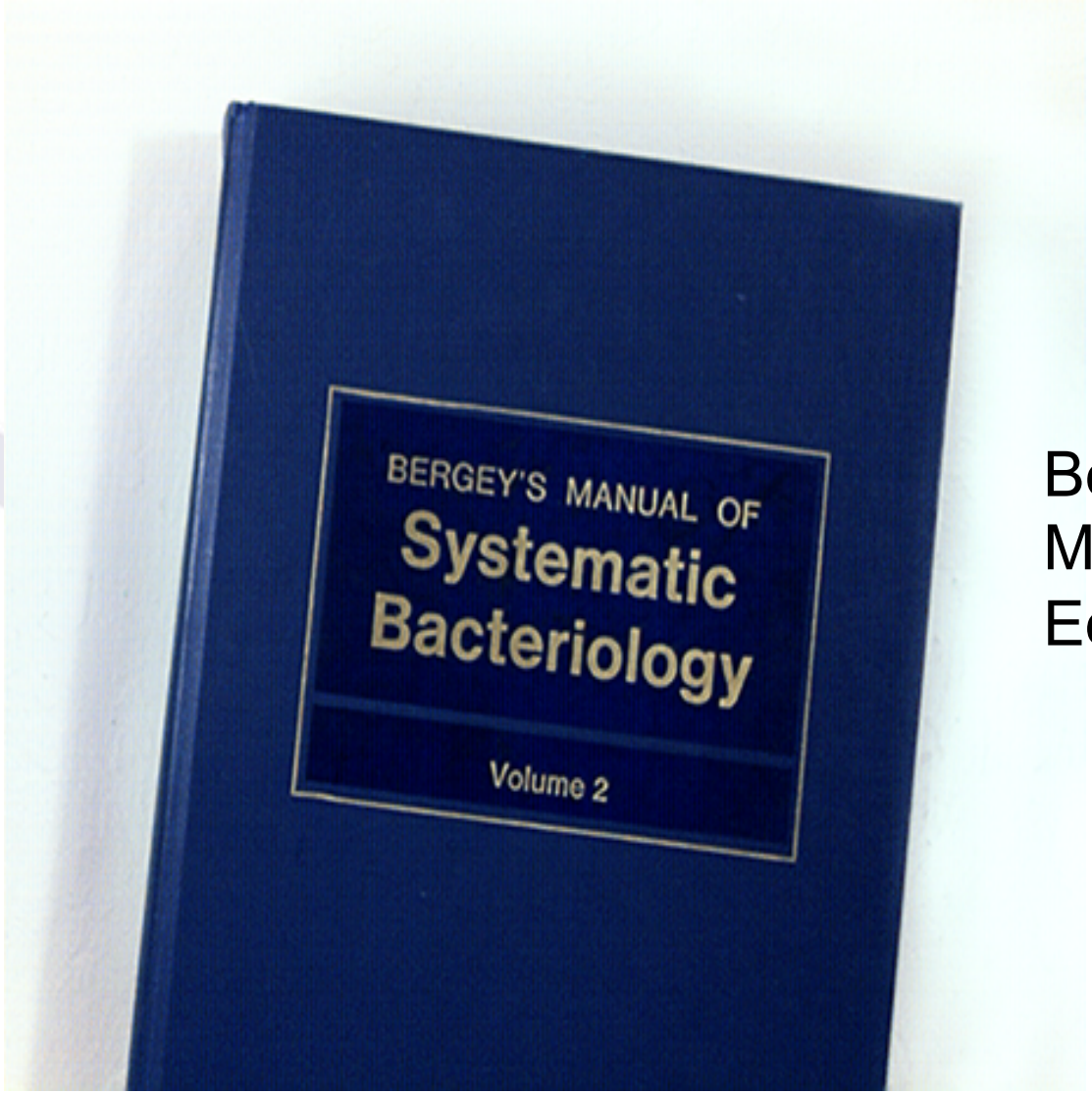
Brief History of Metabolic Phenotypic Analysis

BIOLOG

In the beginning …

The cell was a black box

Early Beginnings of Metabolic Description of Cells



Bergey's Manual 1st Edition, 1923

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L. E. den Dooren de Jong

Survey of C-Source and N-Source Utilization, 1926

B. coli **M. phlei**

TABLE 18[1]

Tapwater with 0·1 % K_2HPO_4, 0·1 % Am_2SO_4, 1 % $CaCO_3$, 0·5 % of the undermentioned compounds	*Bac. vulgatus*	*Bac. mycoides*	*Bac. polymyxa*	*B. aerogenes*	*B. coli*	*B. prodigiosum*	*B. herbicola*	*B. vulgare*	*Myc. phlei*	*Microc. albus*	*Sarc. lutea*	*Ps. fluorescens*	*Ps. aminovorans α*	*Sp. tenue*
Formic acid	—	—	—	—	—	—	—	(—)	—	—	—	(—)		—
Acetic acid	(—)	(—)	—	+	(—)	(+)	(—)	(—)	+	—	+	+	+	(—)
Propionic acid	(—)	(—)	—	—	—	—	(—)	+	+	—	+	+		(—)
Butyric acid	(—)	(—)	—	(—)	—	—	—	(—)	+	—	+	(—)		(—)
Isobutyric acid	(—)	(—)	—	—	—	—	—	(—)	+	—	+	(+)		(—)
Valeric acid	(—)	(—)	—	—	—	—	—	(—)	+	—	+	—	+	(—)
Caproic acid	(—)	(—)	—	(—)	—	+	—	(+)	+	—	+	+		—
Heptylic acid	(—)	—	—	—	—	(+)	—	(+)	+	—	+	+		—
Caprylic acid	—	—	—	—	—	+	—	—	+	—	+	+		—
Nonylic acid	—	—	—	—	—	+	—	—	(+)	—	+	+		—
Capric acid	—	(—)	—	—	—	+	—	—	(+)	—	(—)	+		—
Lauric acid	—	—	—	—	—	—	—	—	—	(—)	—	—		—
Palmitic acid	—	—	—	—	—	—	—	—	—	—	—	—		—
Stearic acid	—	—	—	—	—	—	—	—	—	(—)	—	—		—
Acrylic acid	—	—	—	(—)	—	—	—	—	—	—	+	—		—
α-Crotonic acid	—	—	—	—	—	—	—	—	+	—	(+)	(—)	+	(—)
Undecylic acid	—	—	—	—	—	—	—	(—)	+	—	(+)	(+)	—	—
Oleic acid	(—)	(—)	—	—	—	(+)	—	—	(+)	—	(+)	(+)		—
Elaidic acid	—	—	—	—	—	—	—	—	(—)	—	—	—		—
Glycollic acid	—	—	—	—	—	—	—	—	—	—	—	—	+	—
Lactic acid	(+)	—	—	+	+	+	+	—	+	—	+	+	+	—
α-Hydroxybutyric acid	(—)	—	—	—	—	—	—	—	(—)	—	—	—	+	—
β-Hydroxybutyric acid	—	—	—	—	—	(+)	—	—	(+)	—	(—)	+		(+)
Hydroxyisobutyric acid	—	—	—	—	—	—	—	—	—	—	—	—		—
Glyceric acid	(—)	—	(—)	(+)	(+)	+	(—)	—	(—)	(—)	—	+	+	—

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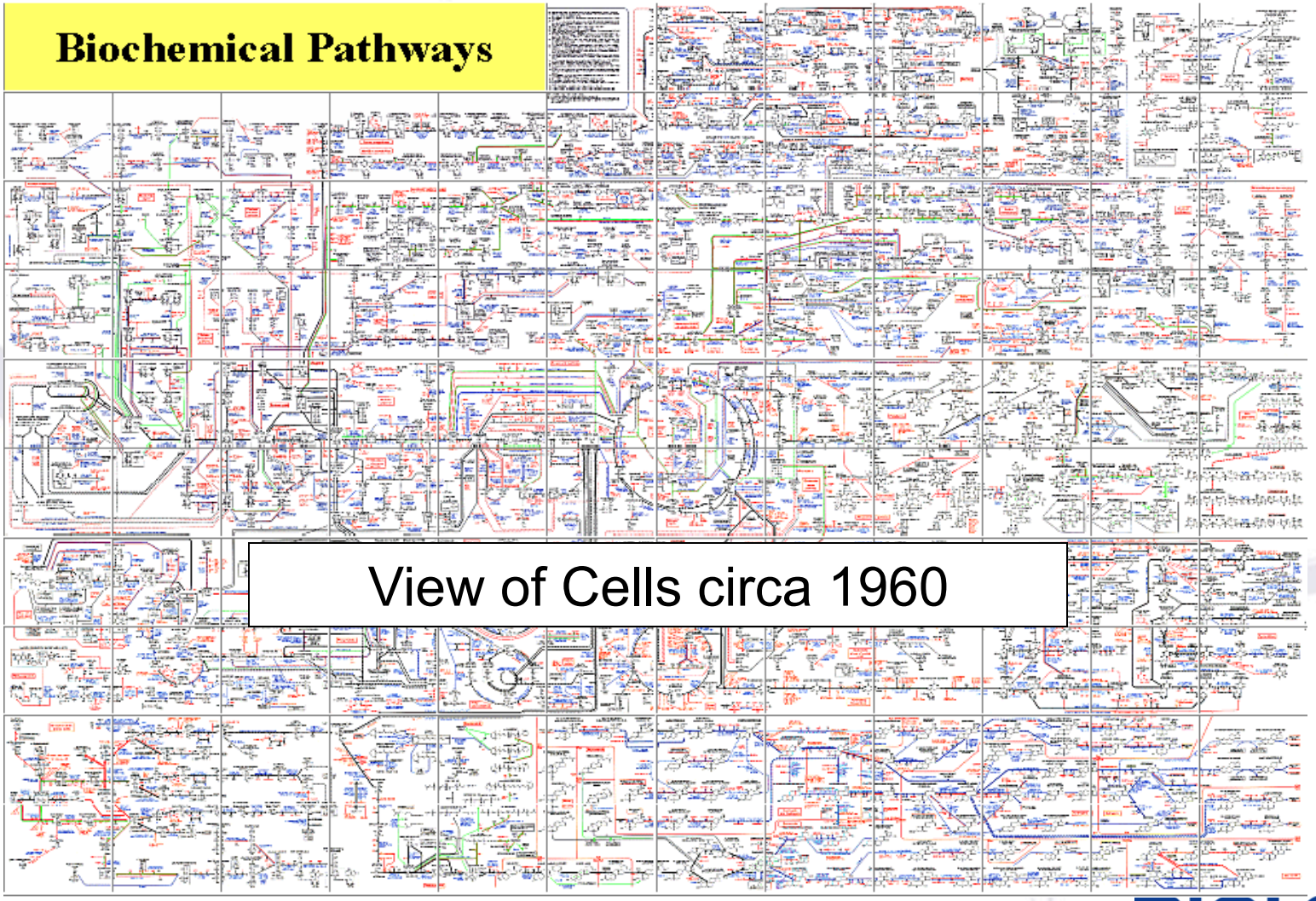
Analogy #1

Metabolic Circuitry Resembles Electronic Circuits



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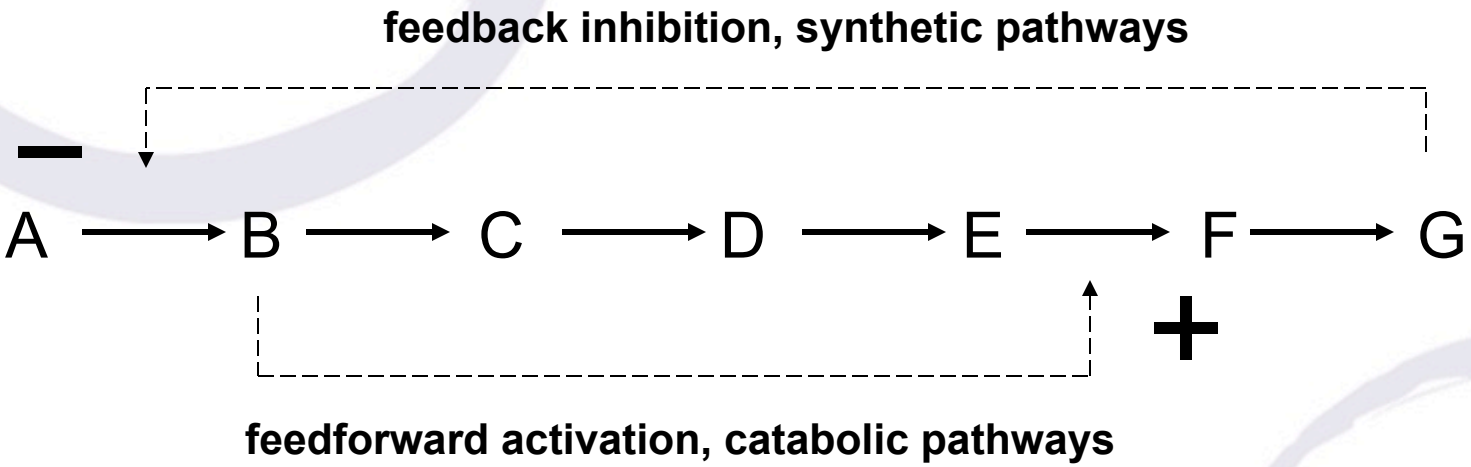
Regulatory Complexity Added to Circuitry, circa 1970



Feedback and feedforward open up the possibility of oscillations

BIOLOG

Metabolic Oscillations

Acta Biochim. et Biophys. Acad. Sci. Hung. Vol. 5 (2), pp. 147—157 (1970)

Altered Repression Behaviour in a Feedback Insensitive Mutant of Escherichia coli K12

L. PATTHY, G. DÉNES

Institute of Medical Chemistry, University Medical School, Budapest, Hungary

(Received January 6, 1970)

One-step mutants showing multiple alterations in the biosynthesis and regulation of the histidine operon were isolated from Escherichia coli K12 on the basis of their resistance to 2-thiazolealanine. The first enzyme of histidine biosynthesis in one of the mutants has lower activity and is partially resistant to inhibition by histidine. The decreased activity of this enzyme necessitates the derepression of the histidine operon in order to produce histidine at a normal rate. Derepression by the mutant, however, can be effected only by severe histidine starvation. This feature is more pronounced at lower temperatures, resulting in cold sensitivity of growth. Thiazolealanine renders derepression and growth normal. The different features of the mutant behaviour may be pleiotropic effects of the mutation in the gene for the first enzyme.

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Metabolic Oscillations

A single gene mutation causes cell growth to oscillate !

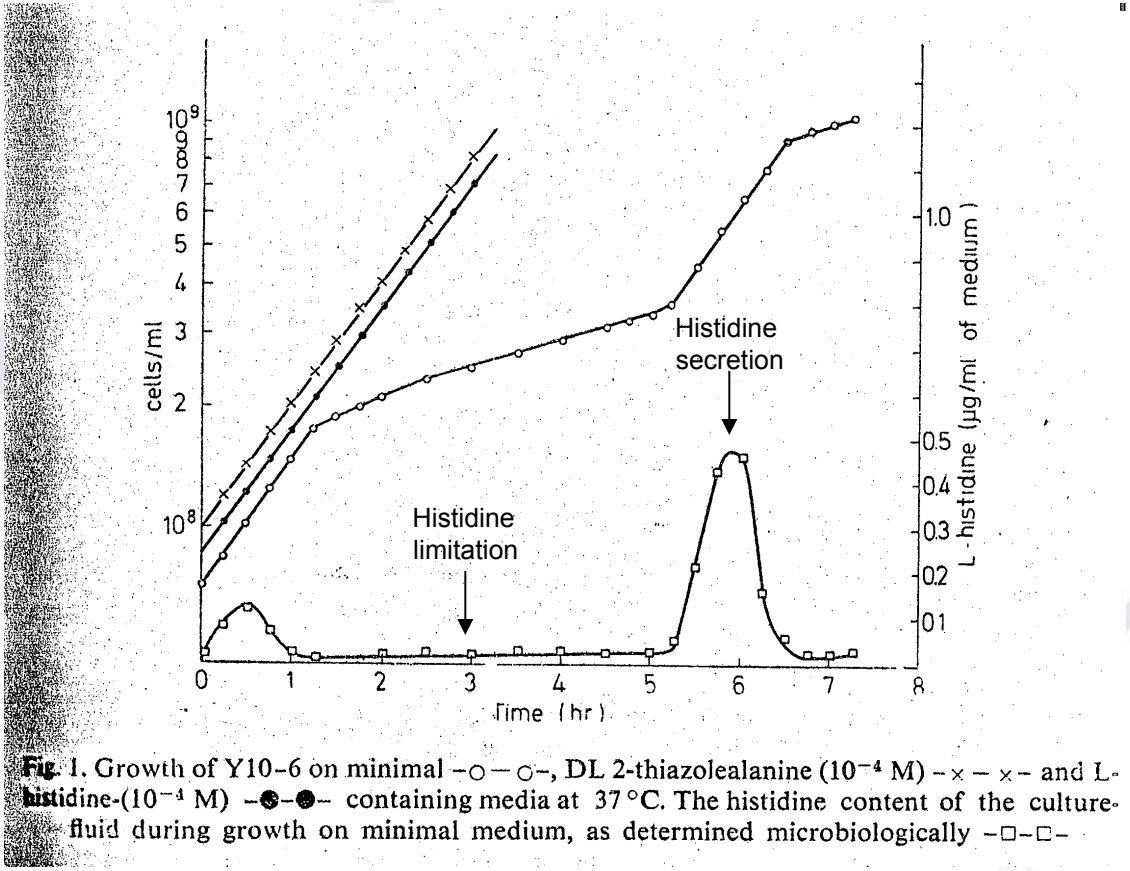

Fig. 1. Growth of Y10-6 on minimal –○—○–, DL 2-thiazolealanine (10^{-4} M) –×—×– and L-histidine-(10^{-4} M) –●–●– containing media at 37 °C. The histidine content of the culture-fluid during growth on minimal medium, as determined microbiologically –□–□–

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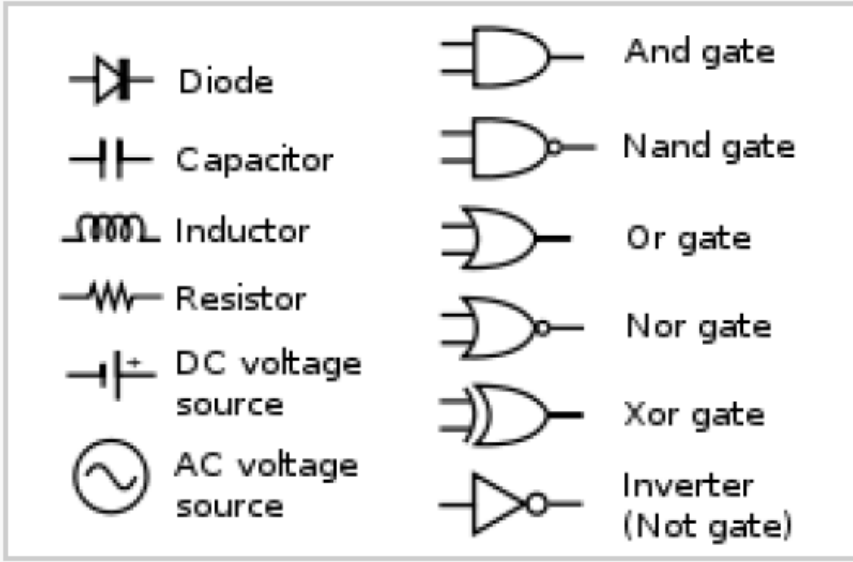
Metabolism Resembles Electronic Circuit Diagrams

Electrical Components



Common schematic diagram symbols (US symbols)

Biological Components

Dehydrogenases	Polymerases
Isomerases	Kinases
Glycosidases	Hydrolases
Phosphatases	Epimerases
Phosphorylases	Transferases
Peptidases	Proteases
Oxidoreductases	Lyases
Aldolases	Ligases
Hydroxylases	Cyclases

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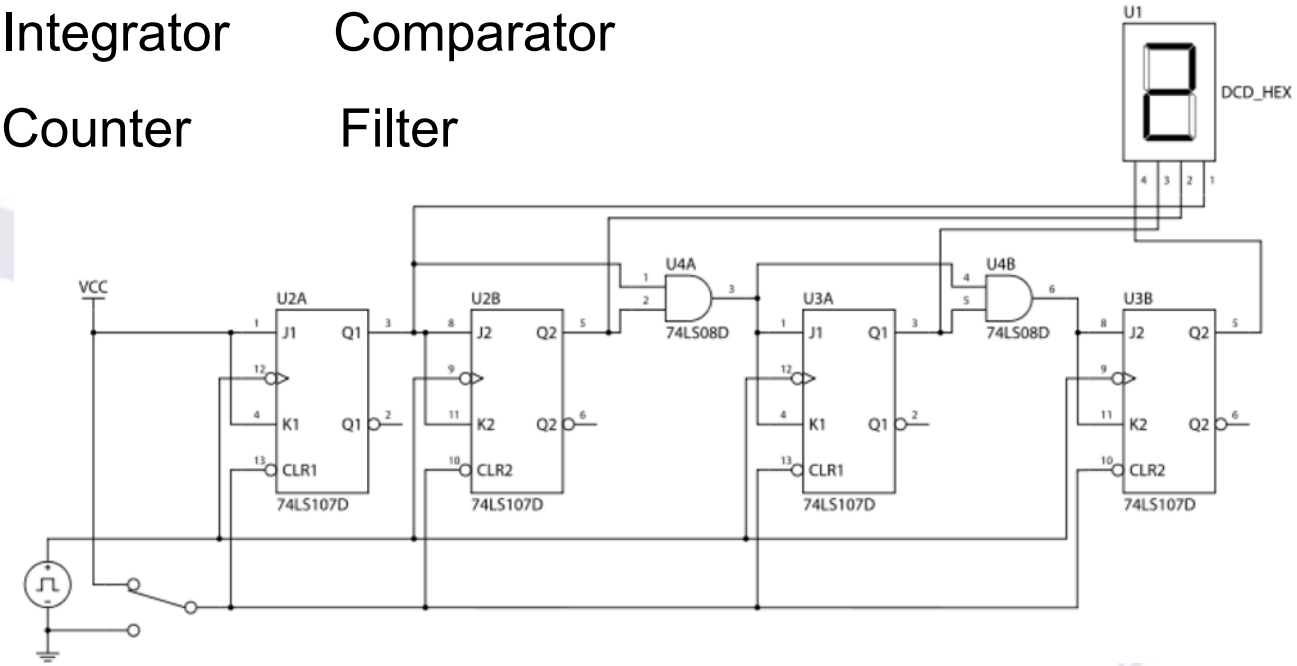
Higher Order Understanding of Electronic Circuits

Amplifier	Receiver
Rectifier	Oscillator
Integrator	Comparator
Counter	Filter



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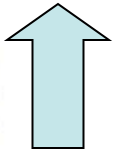
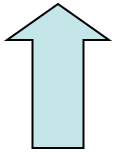
Higher Order Understanding of Cells: Physiology

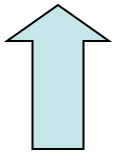
- Growth is a property common to all cells



- Cell growth is primarily polymer synthesis:
  DNA, RNA, protein, membranes, wall, storage polymers



- The polymers are made by assembling subunits:
  deoxynucleotides, ribonucleotides, amino acids, etc.



- The subunits are made from C, N, P, S, O, H

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My Discovery of a Colorimetric Readout of Cell Metabolism - 1975

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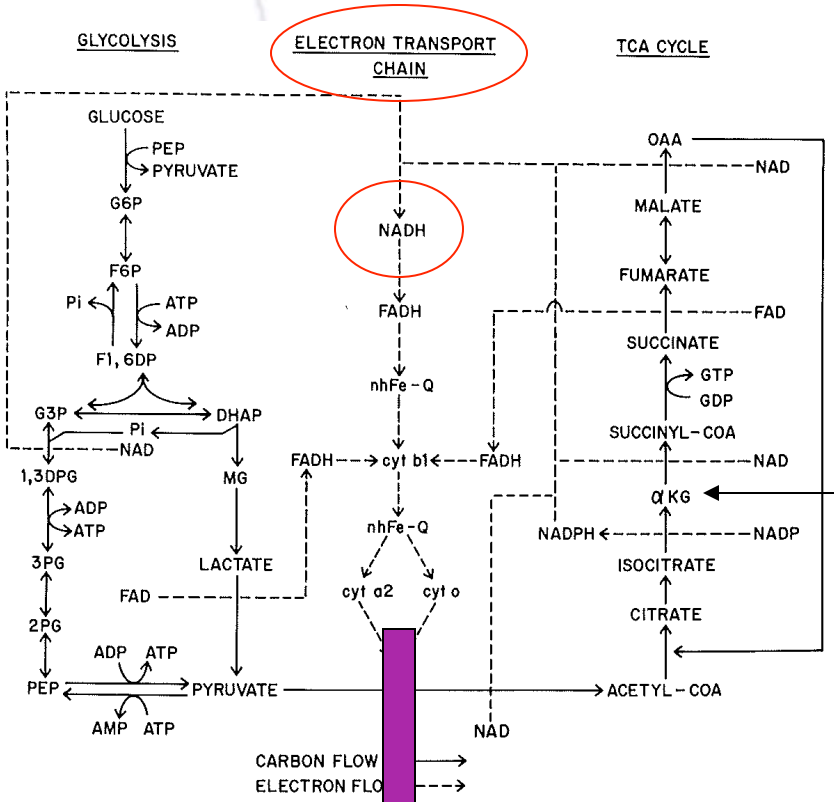
Metabolism of C-sources Produces an Electron Flow



histidine

FIG. 3. *The pathways of central metabolism in* ...*coli and S. typhimurium. The following abbreviations are used: glucose-6-P (G6P), fructose-6-P (F6P), f*...*tose-1,6-diP (F1,6DP), glyceraldehyde-3-P (G3P), 1,3-diP-glycerate (1,3DPG), 3-P-glycerate (3PG), 2-P-g*...*erate (2PG), P-enolpyruvate (PEP), dihydroxyacetone-P (DHAP), methyl glyoxal (MG), non-heme iron-*...*yme Q complex (nhFe-Q), cytochrome (cyt), oxaloacetate (OAA), and α-ketoglutarate (αKG).*

Redox Dye

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Using a Redox Dye to Detect Metabolic Flux

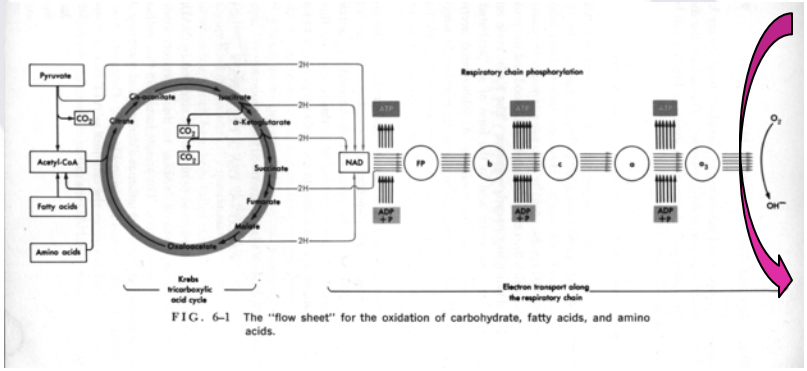
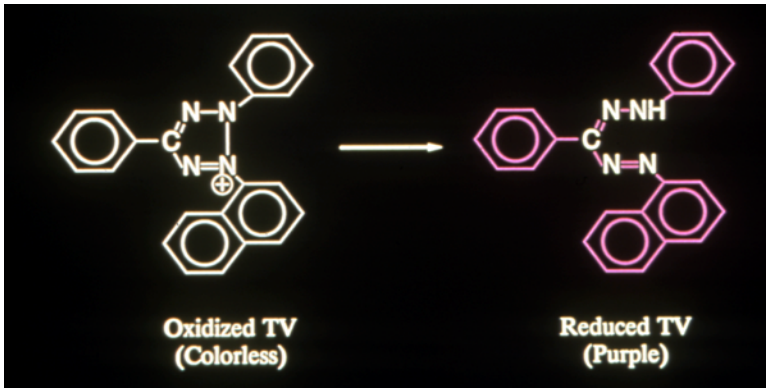


FIG. 6-1 The "flow sheet" for the oxidation of carbohydrate, fatty acids, and amino acids.

TV_{ox}

TV_{red}



Biolog uses a redox reporter dye that detects energy (NADH) production

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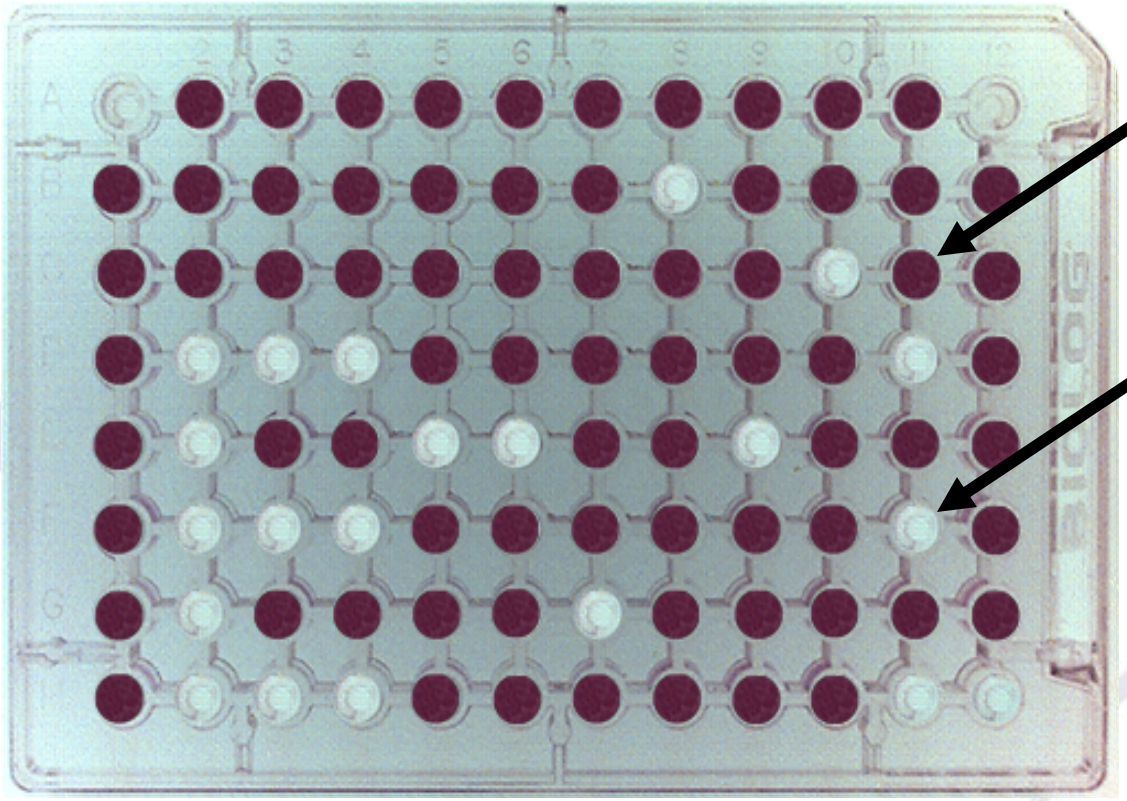
Redox Chemistry Measures Cell Energetics

Microplate containing a negative control well and 95 different carbon substrates



Add cells → Add redox dye

Stimulatory chemicals enhance energy production

inhibitory chemicals block energy production

Wells contain different tests and measure different pathway activities and phenotypes of cells

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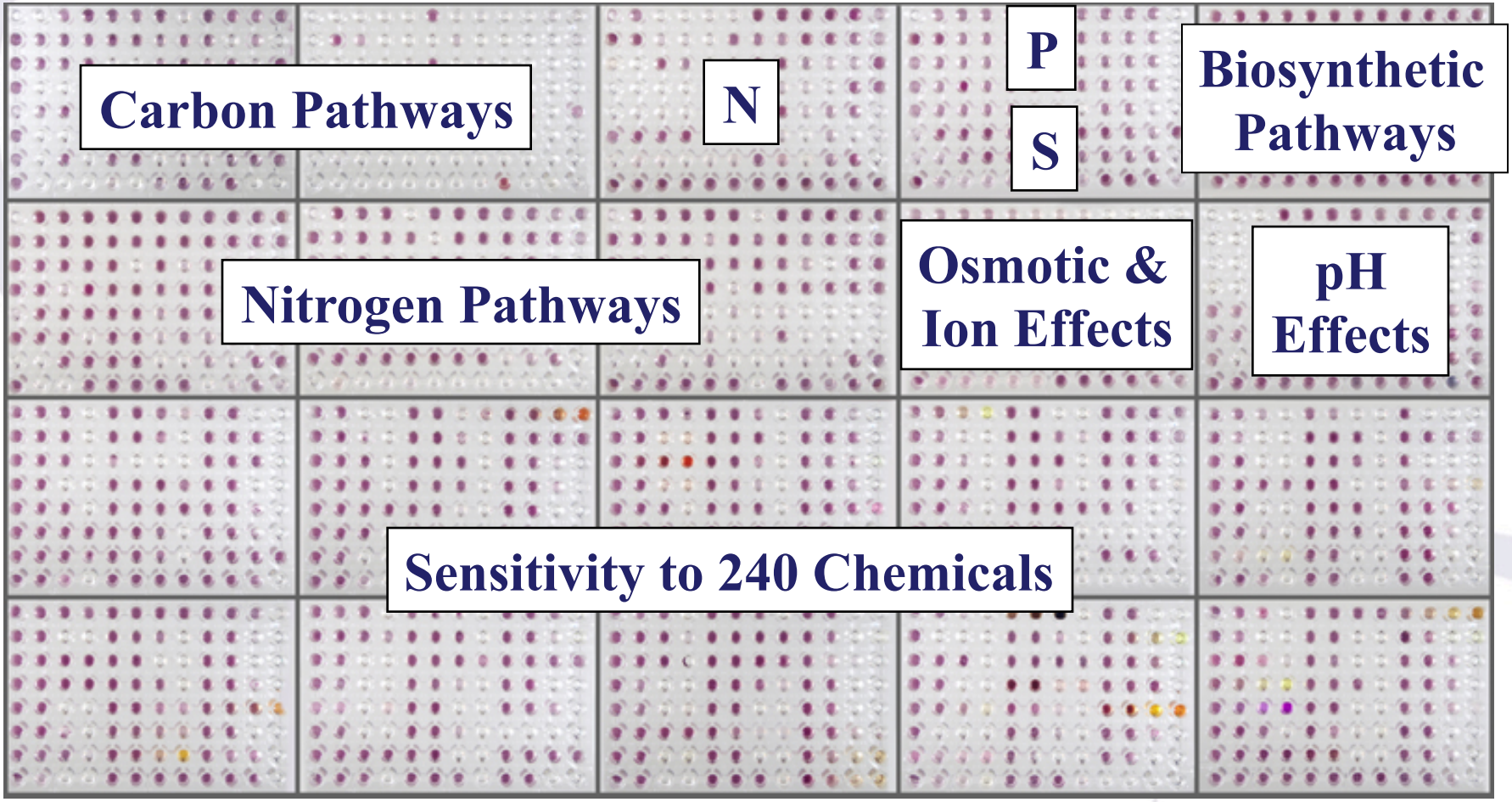
PM Platform - ~2,000 Phenotypic Assays, circa 2000



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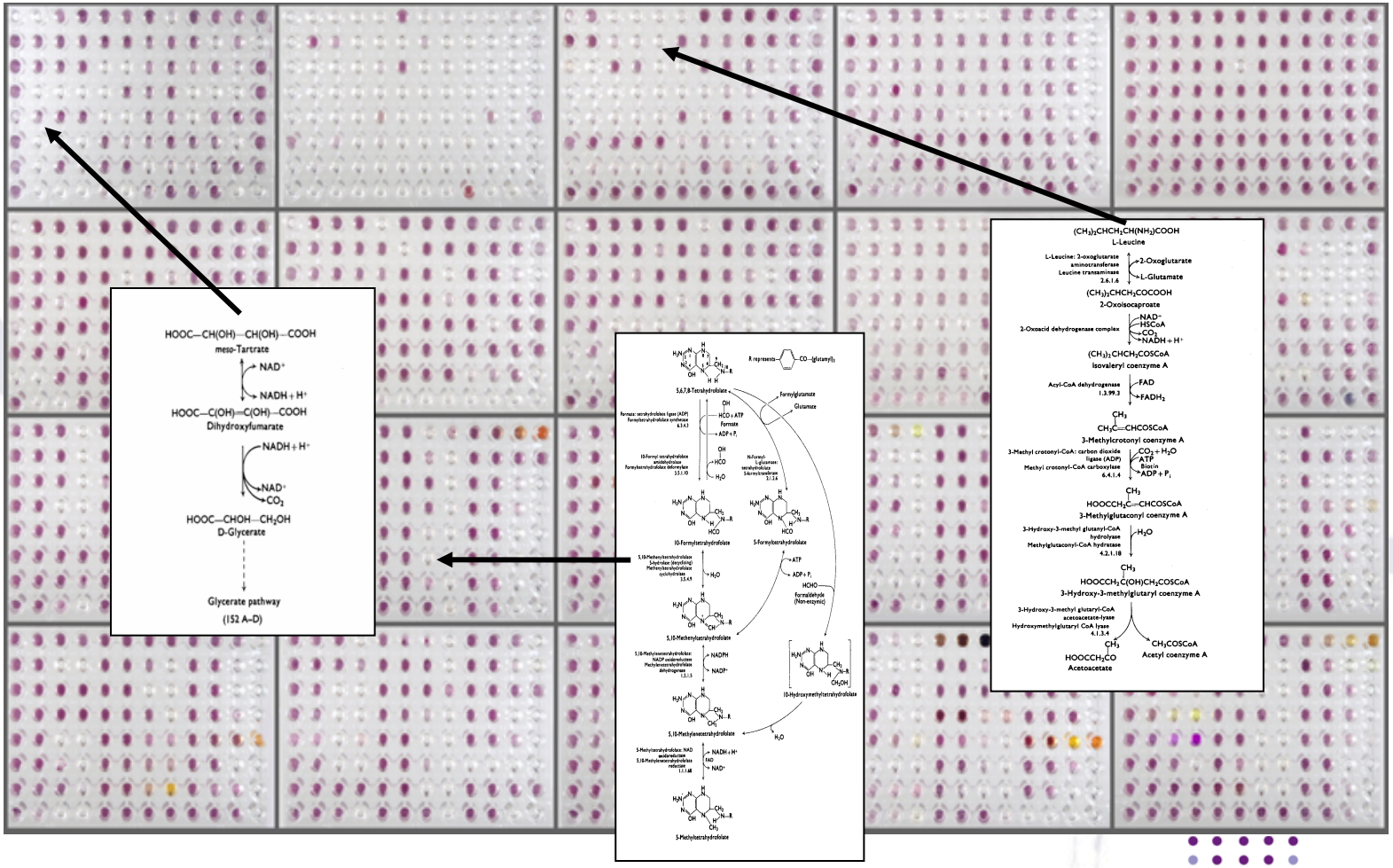
PM Platform - Pathway Readout

complete medium

- C
- N
- P
- S
- K
- Na
- Mg
- Ca
- Fe
- aa
- vit
- +inh



It is like having a flux meter to measure individual pathways

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Analogy #2

The Cell Resembles a Signal Processor

From a Redox Color Change to Scanning Cell Physiology

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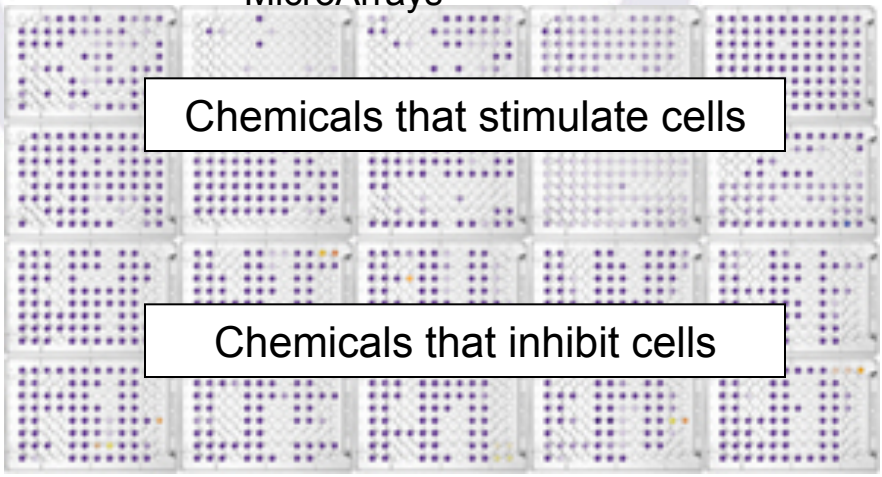
2 Components of the PM Cell Assay Platform

Phenotype MicroArrays™



colorimetric cell assays in 96-well microplates

OmniLog™ Incubator/Reader

incubation and recording of data in the OmniLog

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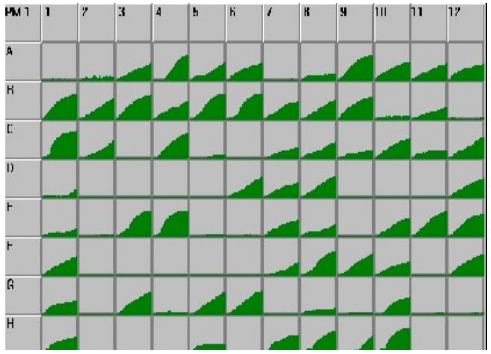
PM Assays are Easy to Run

Assays Initiated by adding cells to wells

100 µl per well

OmniLog PM System

Holds 50 microplates at a set temperature and measures color formation at 15-minute intervals



Kinetic assay readout for up to 5,000 wells

CVs typically < 10%

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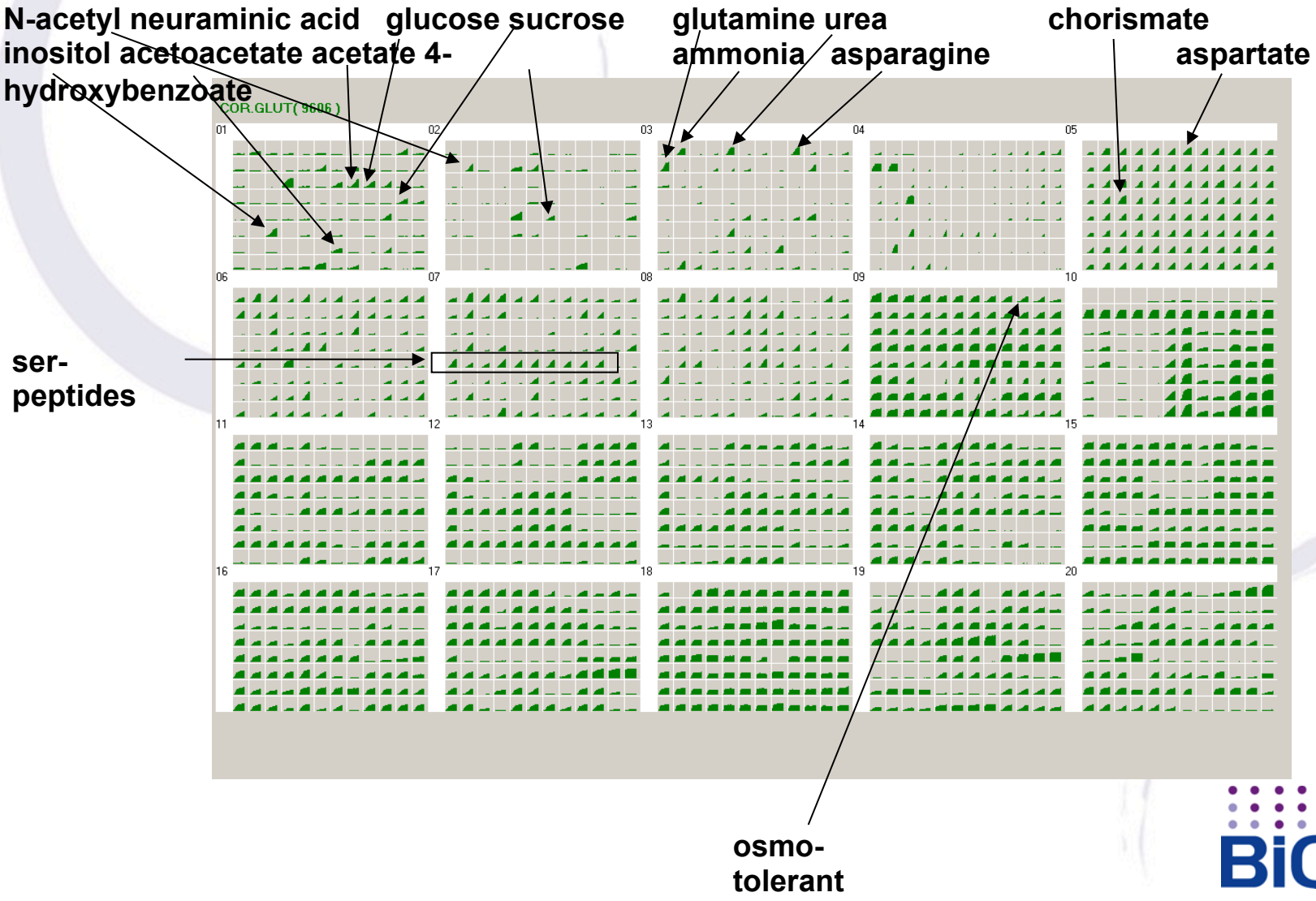
PM Analysis of Corynebacterium glutamicum



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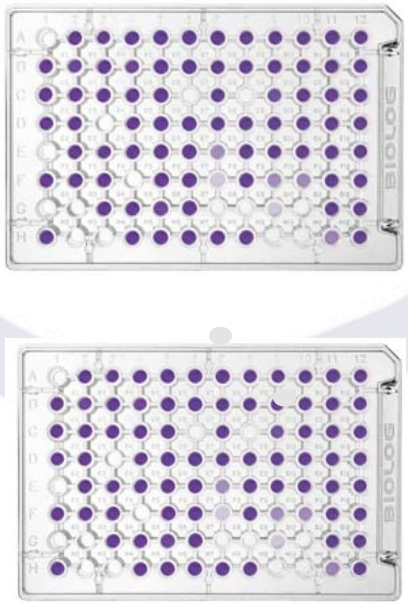
PM Platform - Comparing Two Cell Lines

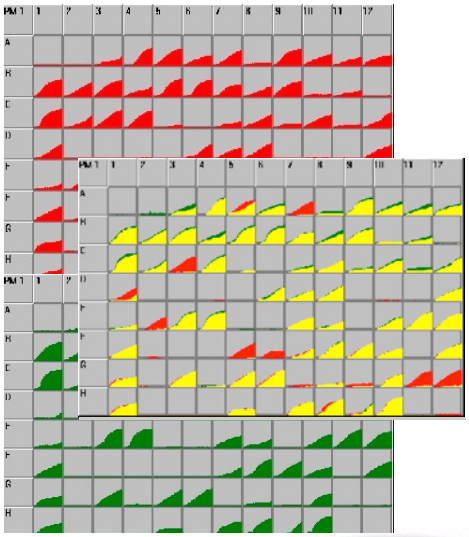
Add cell **A**

Add cell **B**



PM Pattern

OmniLog PM System



PM Kinetic Result

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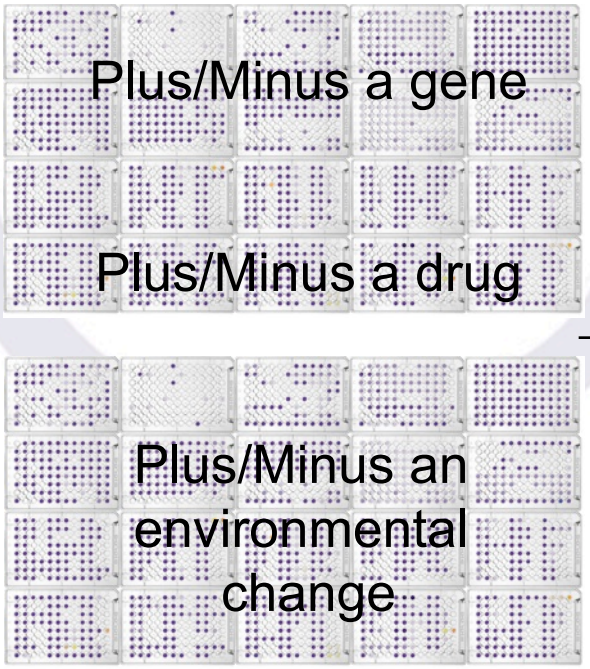
PM Platform – Comparing Two Assay Conditions

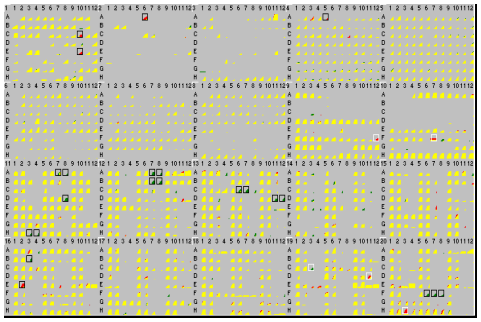


PM Pattern

1 hr

OmniLog PM System

Automatic



PM Kinetic Result

24-48 hr

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Analyzing Gene Function: Metabolic Genes and Drug Resistance Genes

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E. coli malF::Tn10 vs MG1655

Dextrin

Maltose

Maltotriose

tetracyclines

tetracyclines

	Name	Strain Number	Other	
Test	EP005	MG1655 malF3089::Tn10		
Ref	MG1655FB	1998 version	E.coli	
Phenotypes Gained - Faster Growth / Resistance				
PM	Wells	Test	Difference	Mode of Action
PM16	B 3	Norfloxacin	75	DNA topoisomerase, quinolone
PM20	F 6, F 7, F 8	Oxytetracycline	239	protein synthesis, tetracycline
PM12	B 7, B 8	Penimepicycline	207	protein synthesis, tetracycline
PM13	D 11, D 12	Rolitetracycline	183	protein synthesis, tetracycline
PM12	A 7, A 8	Tetracycline	182	protein synthesis, tetracycline
PM13	C 6, C 7	Doxycycline	177	protein synthesis, tetracycline
PM11	D 8	Demeclocyline	104	protein synthesis, tetracycline
PM11	A 7, A 8	Chlortetracycline	94	protein synthesis, tetracycline
PM11	H 3, H 4	Cephalothin	127	wall, cephalosporin
Phenotypes Lost - Slower Growth / Sensitivity				
PM	Wells	Test	Difference	Mode of Action
PM02	A 6	Dextrin	-100	C-source
PM01	E 10	Maltotriose	-89	C-source
PM01	C 10	Maltose	-78	C-source
PM04	A 5	Tripolyphosphate	-63	P-source
PM16	E 2	Streptomycin	-133	protein synthesis, aminoglycoside

tetracycline s

Red = Phenotypes Lost **Green = Phenotypes Gained**

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Analyzing Regulatory Genes

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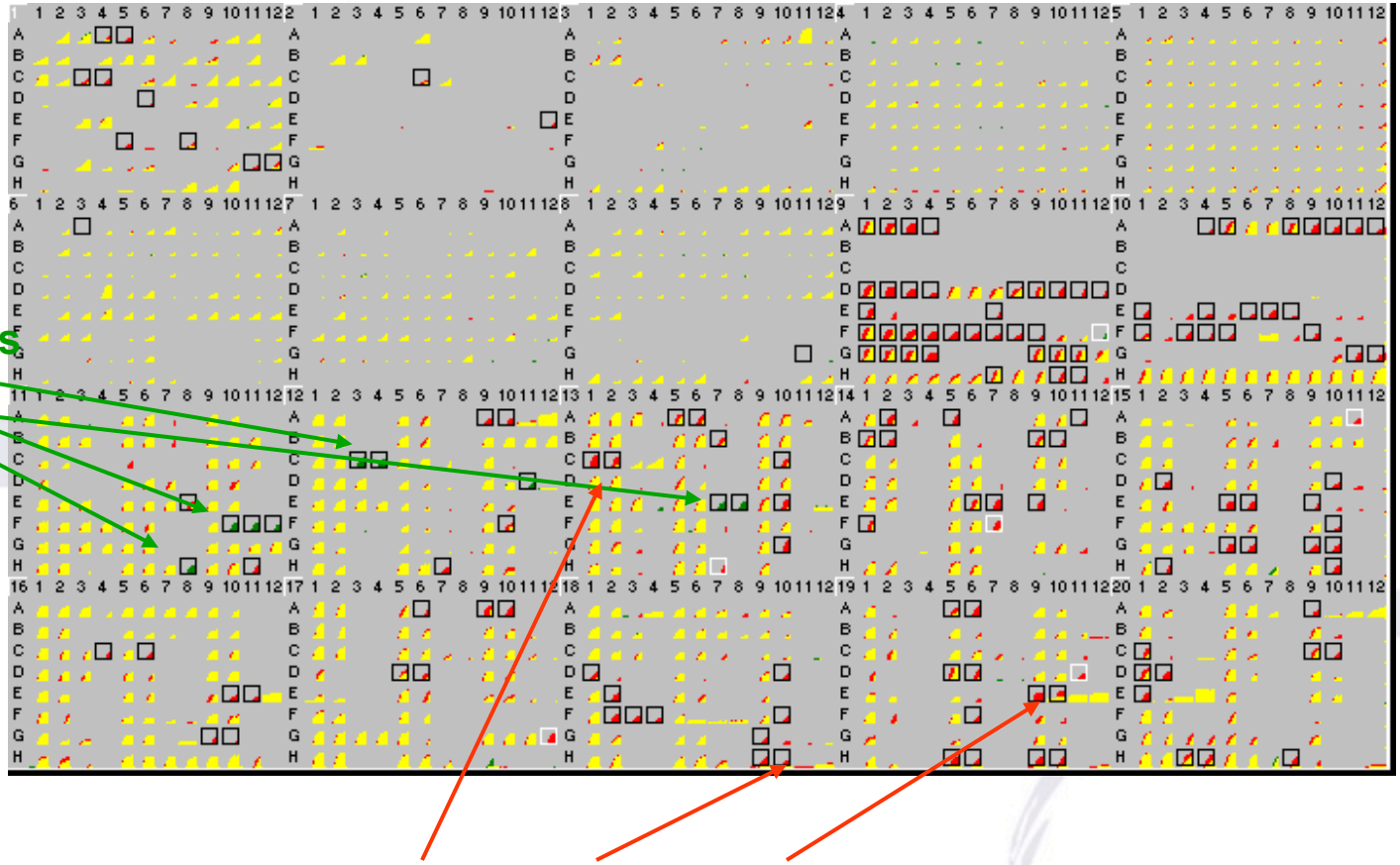
E. coli oxyR::kan vs MG1655

amino-glycosides



t-butyl hydroquinone, plumbagin, lawsone

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Analyzing Genes of Unknown Function

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E. coli b1012 Operon is Regulated by NtrC

b1006-b1012

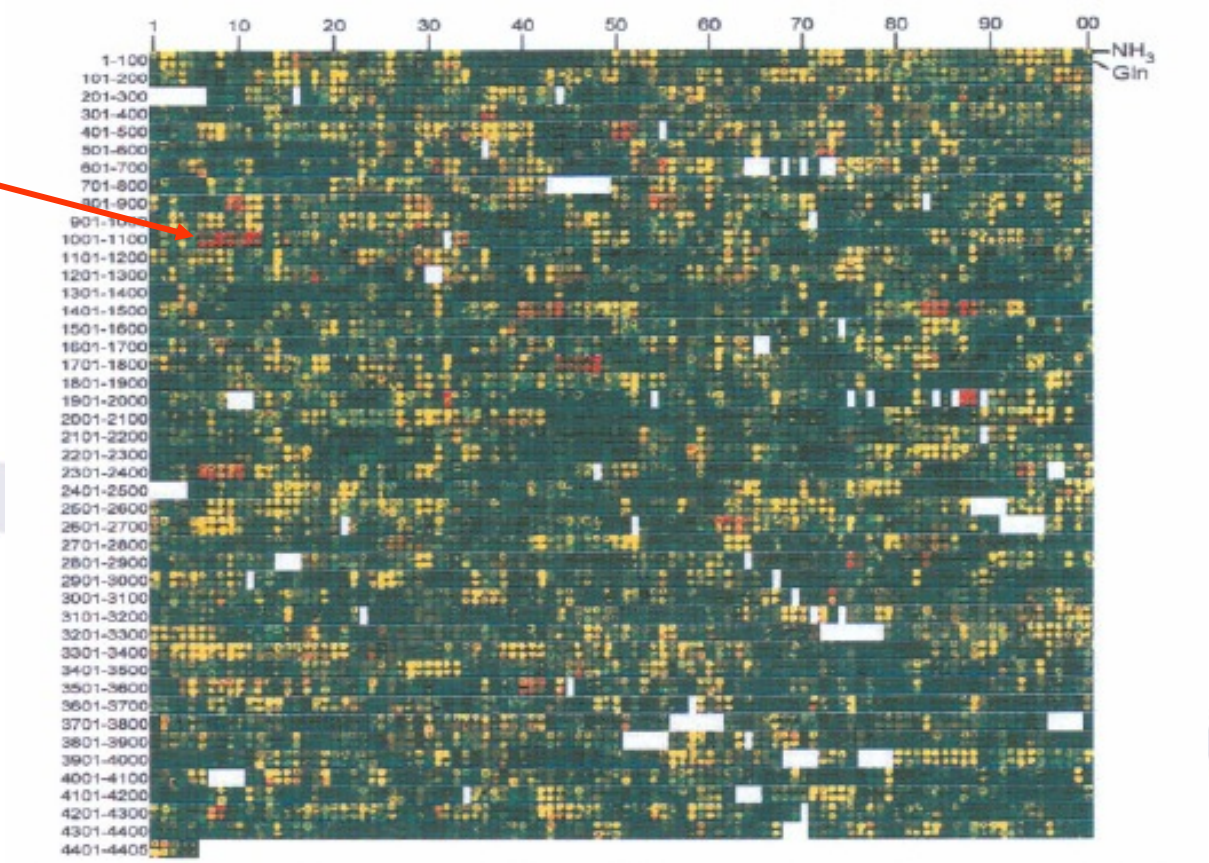

Fig. 1. Two aligned genome images. Microarrays were probed with mixtures of cDNAs from the *glnL*(Up) and *glnG* strains grown on ammonium (upper row of each pair, experiment *c*) or glutamine (lower row, experiment *e*) as nitrogen source. Spots from fluorescence scanning images of microarrays were rearranged in genome order. The *b* number centuries (6) are indicated to the left. Blanks represent either *b* numbers that do not correspond to ORFs or that no longer exist. Red spots can be seen for most operons in Table 1. For some highly expressed genes—e.g., *coaBA* (b0336–37) in the upper row and *glnA* (b3870)—spots appear intense yellow rather than red because of image saturation.

Low, Kustu, and coworkers PNAS (2006) 103:5114

BIOLOG

PM Analysis of Changes in N-metabolism

Nitrogen Metabolism E. coli b1012 Operon Knockout, 25˚C

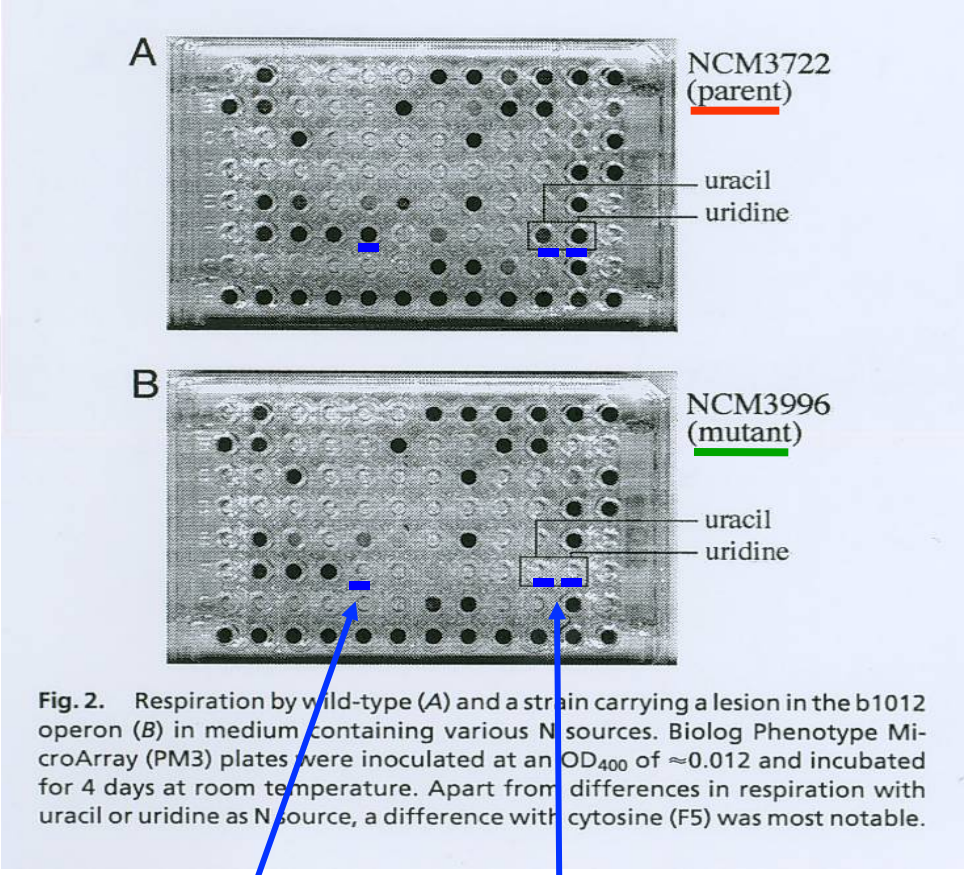

Fig. 2. Respiration by wild-type (*A*) and a strain carrying a lesion in the b1012 operon (*B*) in medium containing various N sources. Biolog Phenotype MicroArray (PM3) plates were inoculated at an OD_{400} of ≈0.012 and incubated for 4 days at room temperature. Apart from differences in respiration with uracil or uridine as N source, a difference with cytosine (F5) was most notable.

cytosine

uracil, uridine

The b1012 operon was noted on E. coli gene chips to be highly regulated by the ntrC (glnG) system. Homology data for b1006 indicated similarity to a nucleobase transporter.

Low, Kustu, and coworkers PNAS (2006) 103:5114

BIOLOG

New Pyrimidine Catabolic Pathway Discovered

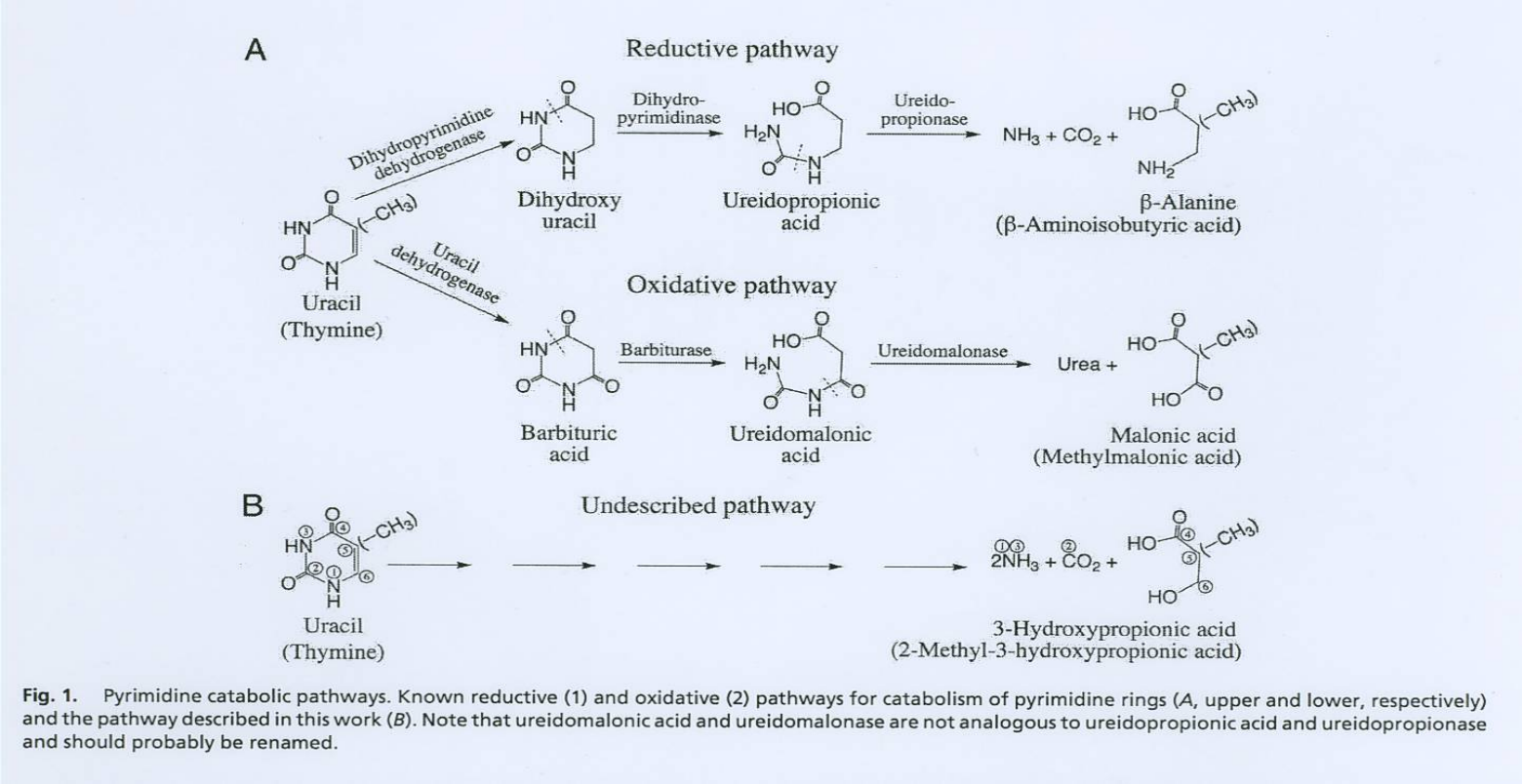

Fig. 1. Pyrimidine catabolic pathways. Known reductive (1) and oxidative (2) pathways for catabolism of pyrimidine rings (*A*, upper and lower, respectively) and the pathway described in this work (*B*). Note that ureidomalonic acid and ureidomalonase are not analogous to ureidopropionic acid and ureidopropionase and should probably be renamed.

Low, Kustu, and coworkers PNAS (2006) 103:5114

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Analyzing Regulation of Metabolism

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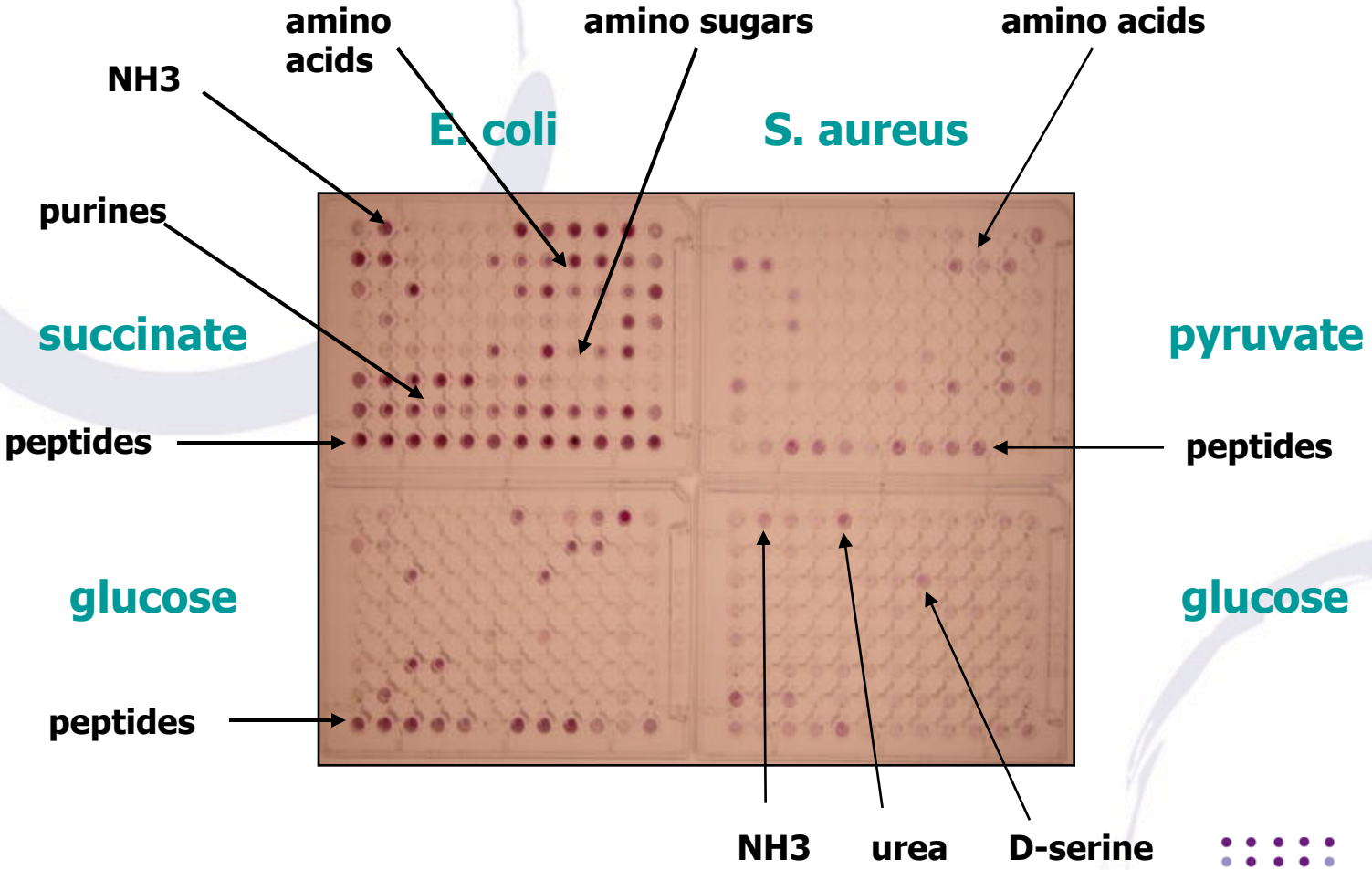
Coordination of N-Metabolism with C-Metabolism

Biolog N-Source plate (PM3) tested with different C-Sources



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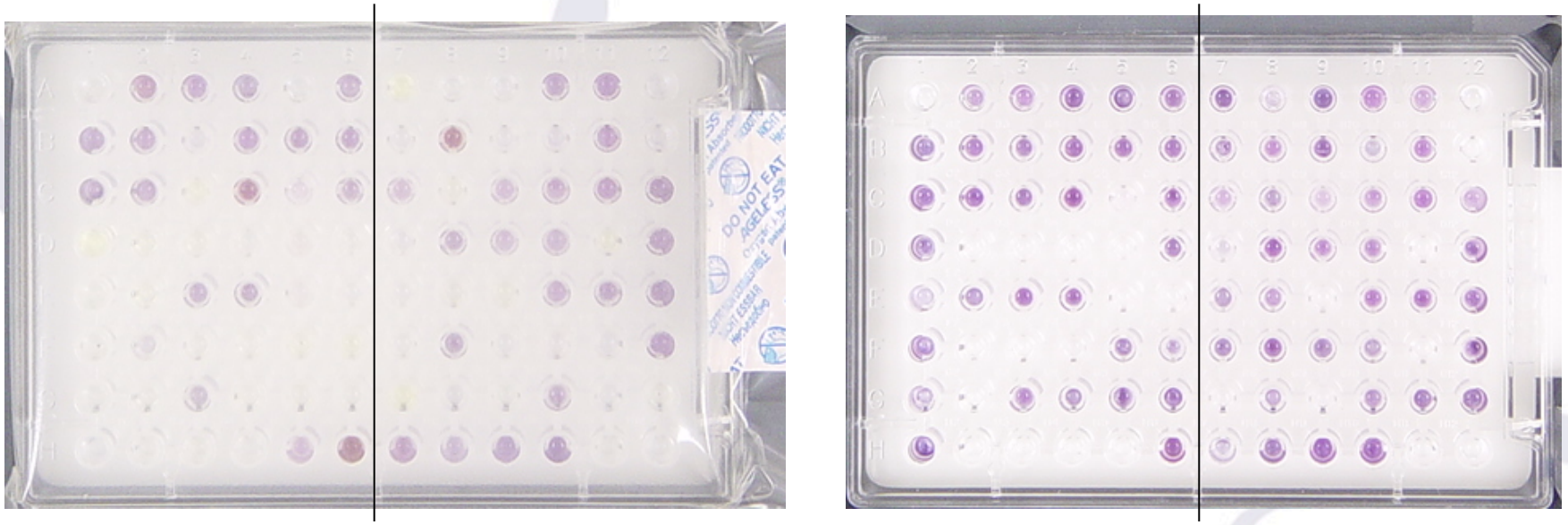
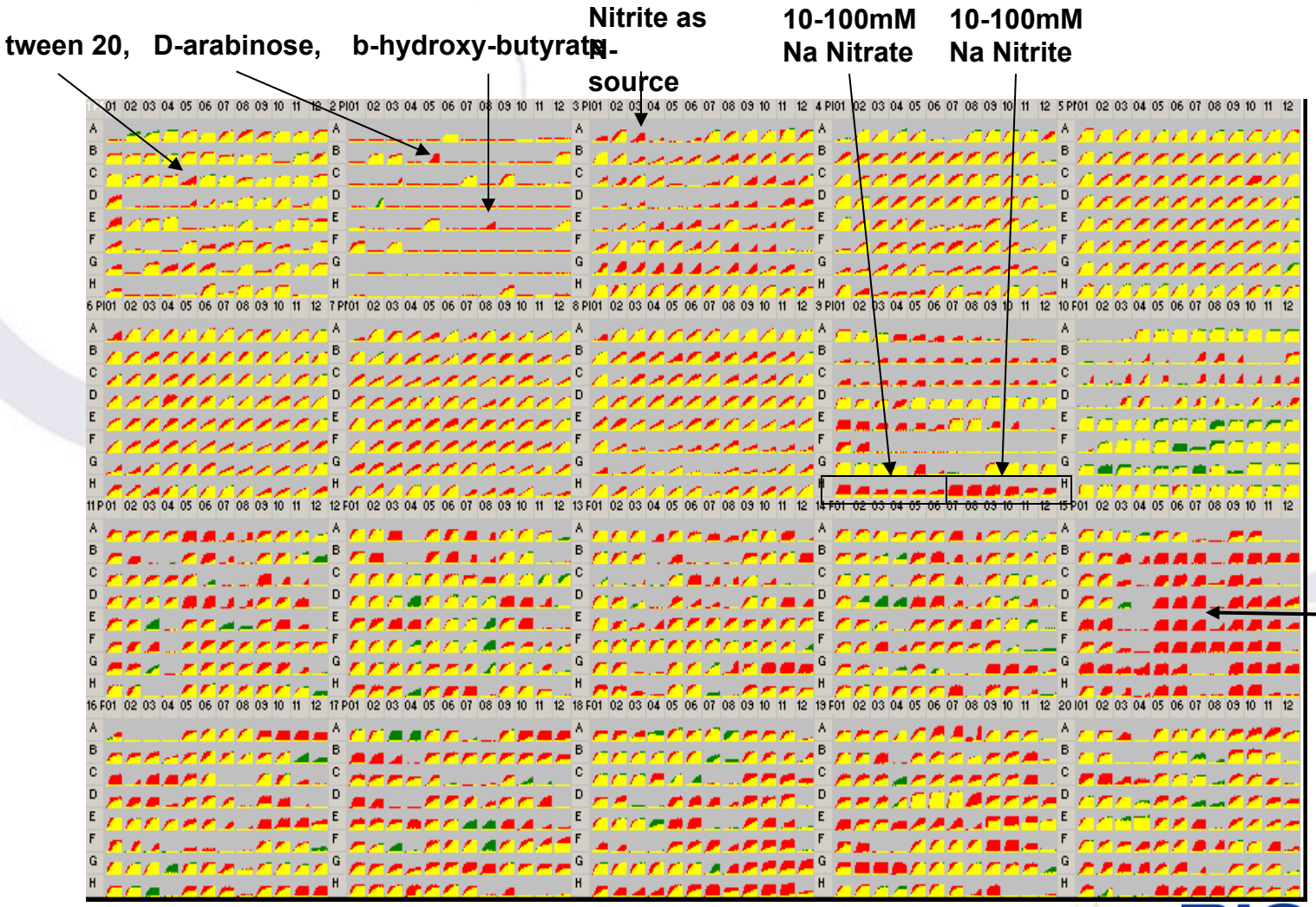
Oxygen Effects on E. coli C-Metabolism

E. coli BW30270 anaerobic (left) vs aerobic (right)
PM1 incubated for 46 hours at 36° C



Under anaerobic conditions, the following C-sources are not metabolized:
A5= succinic acid, A7= L-aspartic acid, A9= D-alanine, B3= glycerol, B7= a-glycerol-PO4, B9= L-lactic acid, B10= formic acid, C3= D,L-malic acid, C8= acetic acid, D1= L-asparagine, D6= a-keto-glutaric acid, E1= L-glutamine, E2= m-tartaric acid, E6= a-hydroxy-glutaric acid lactone, E7= a-hydroxy-butyric acid, F1= glycyl-L-aspartic acid, F5= fumaric acid, F6= bromo-succinic acid, F7= propionic acid, F9= glycolic acid, F10= glyoxylic acid, G1= glycyl-L-glutamic acid, G4= L-threonine, G5= L-alanine, G6= L-alanyl-glycine, G8= N-acetyl-b-D-mannosamine, G11= D-malic acid,

pH Effects on E. coli: pH7 vs pH5



at acidic pH, $NO_3^- \rightarrow NO_2^- \rightarrow HNO_2$ (nitrous acid) and NO (nitric oxide)

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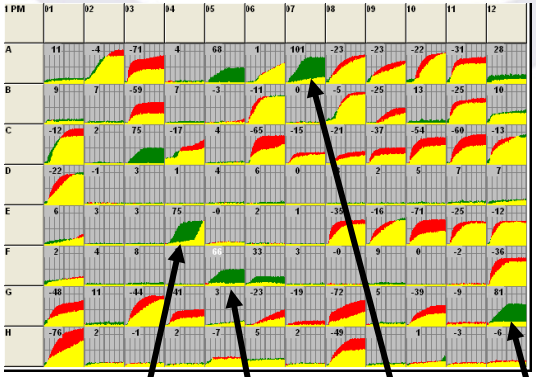
Temperature Effects on C-Metabolism

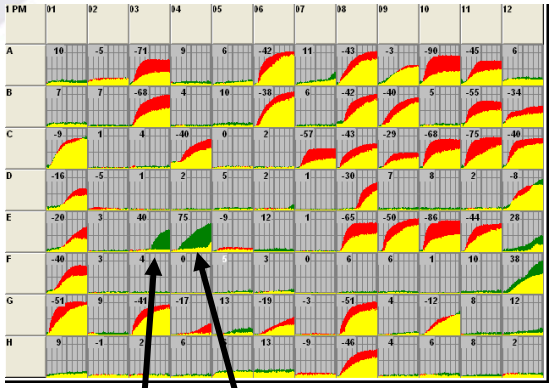
Yersinia pseudotuberculosis strains: 26°C vs 33°C

1087



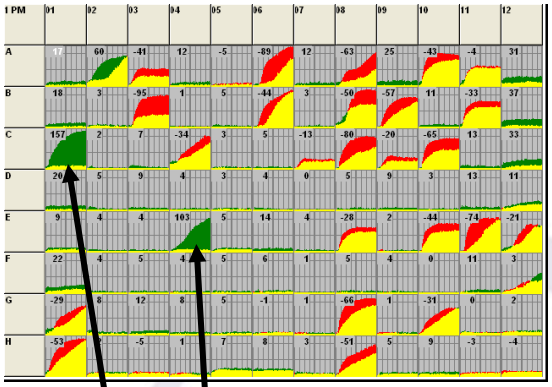
F6P **fumarate** **aspartate** **malate**

15464 (type)



G1P **F6P**

15478



G6P **F6P**

Recent results show that Yersinia has a temperature sensing protein, RovA, that is an important regulator of pathogenicity

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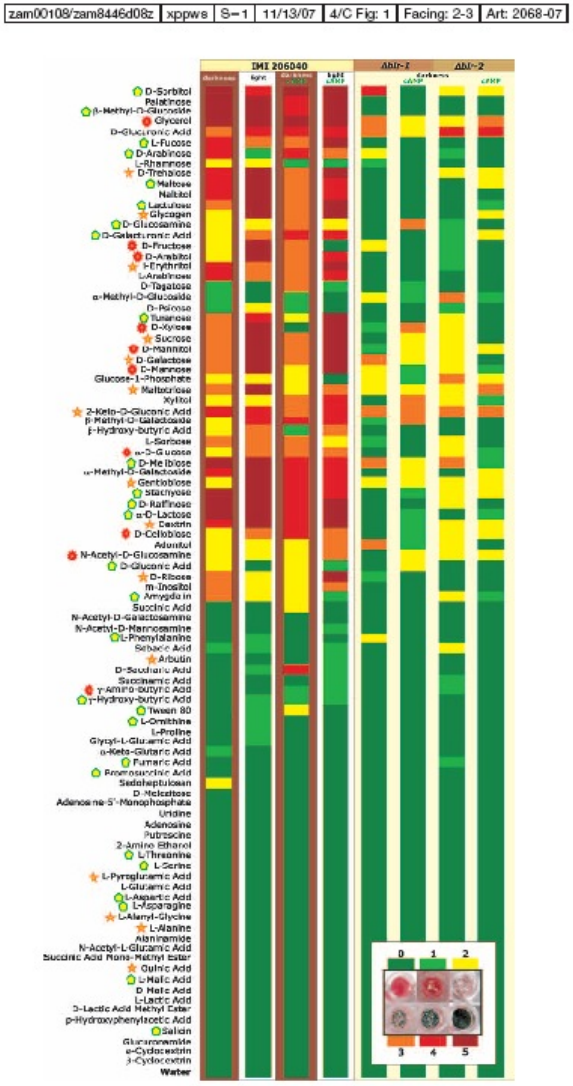
Light and C-Source Effects on Conidiation



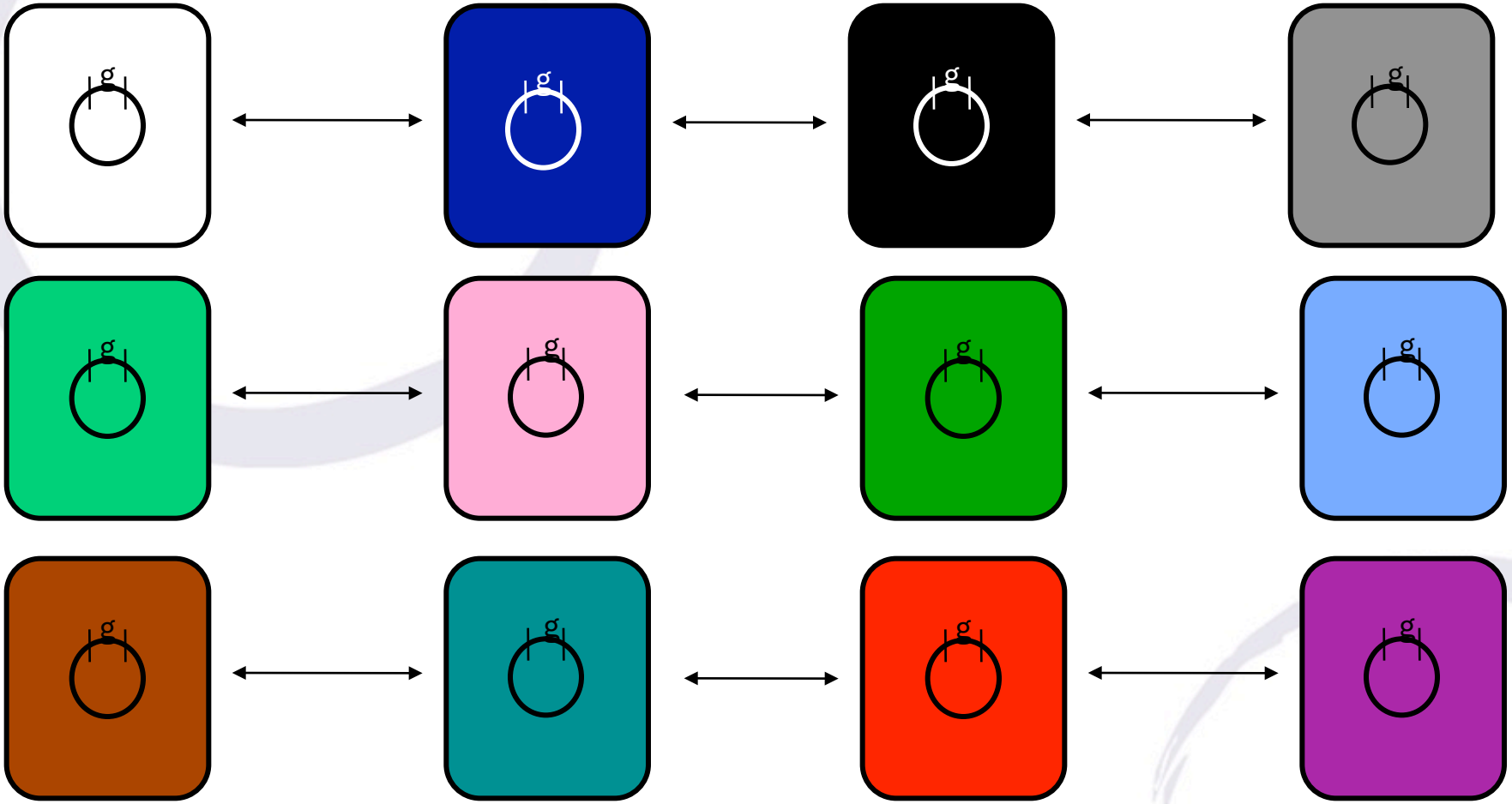
Freidl, MA, Kubicek, CP, and Druzhinina, IS, Applied Environ. Micro. Jan. 2008.

Using the fungus Hypochrea atroviridis, which is a model organism for both cellulose degradation and photomorphogenesis, the authors showed that, contrary to common dogma, C-source has a much more profound effect on conidiation than light exposure.

BIOLOG

Analogy #3

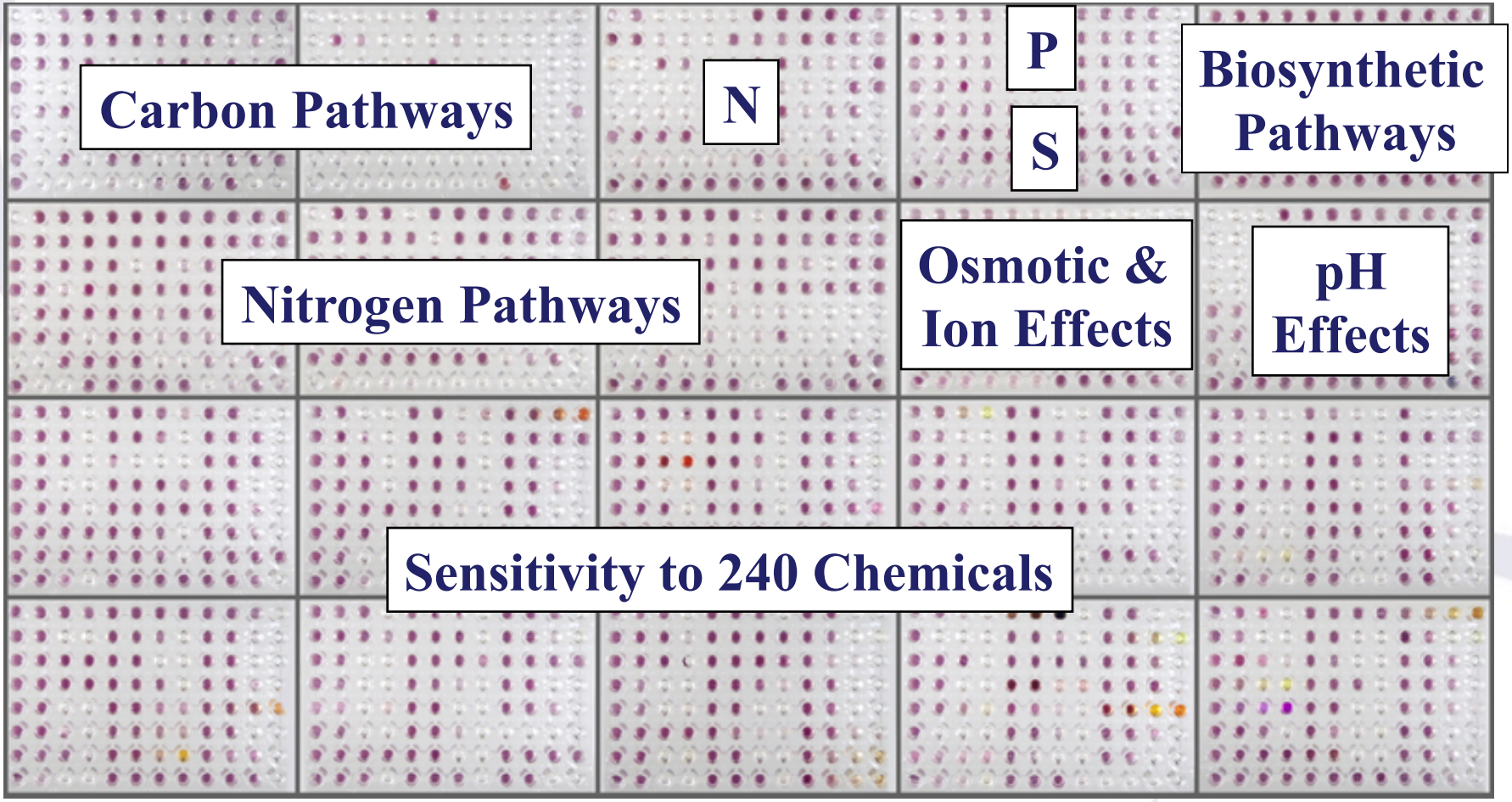
Cells are Multi-State Automata



All Cells Change with Culture Conditions

BIOLOG

PM Platform - ~2,000 Culture Conditions

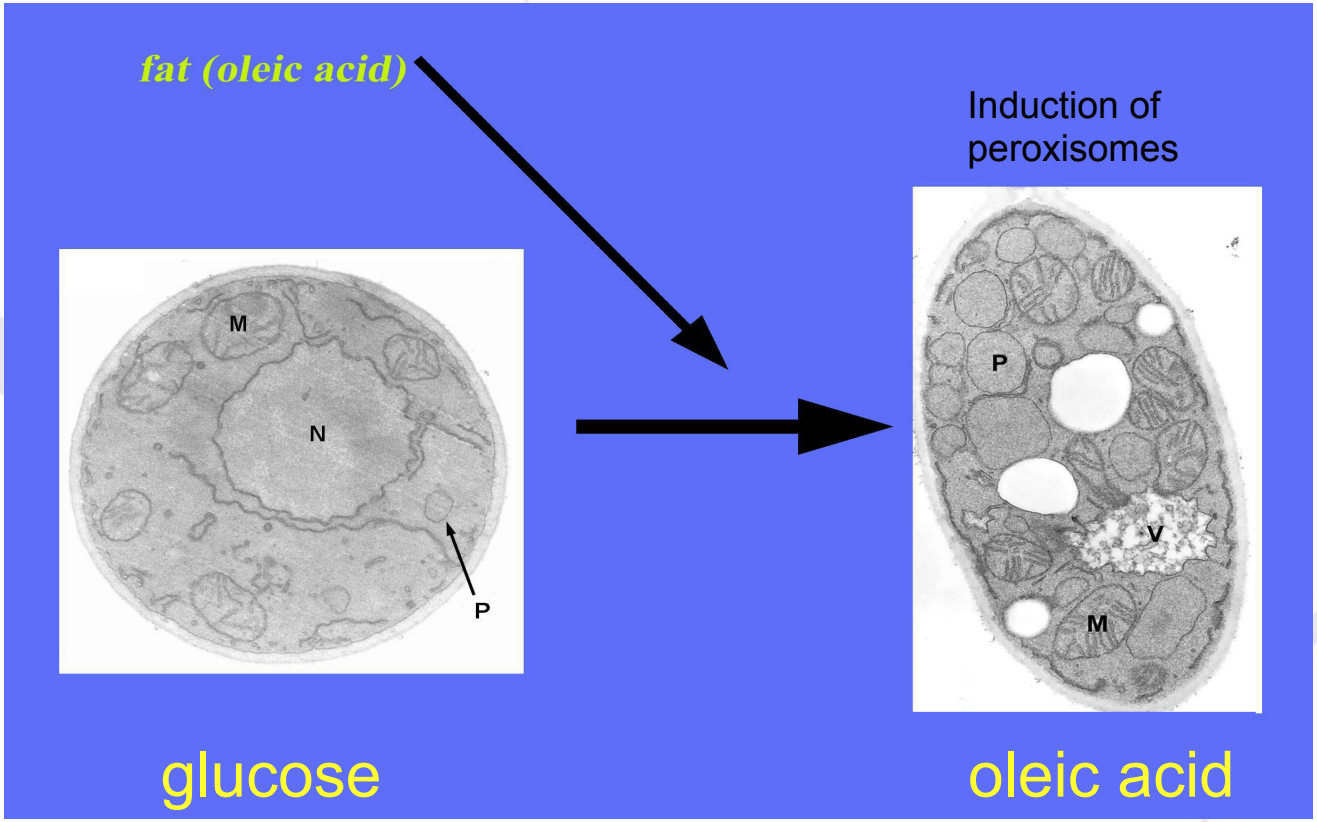


2,000 Versions of the Cell

BIOLOG

Changes in S. cerevisiae with Culture Conditions

Induced by Growth on Different Carbon Sources



Slide generously provided by Richard Rachubinski

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Changes in C. albicans with Culture Conditions

Non-pathogenic form

Pathogenic form

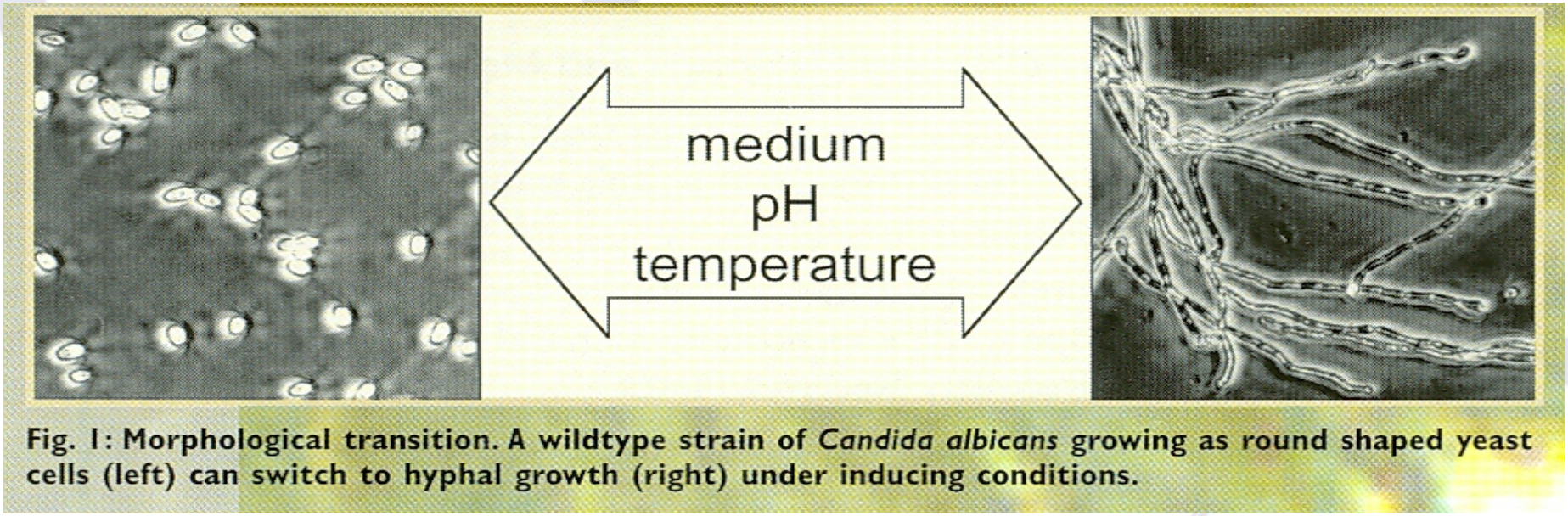


Fig. 1: Morphological transition. A wildtype strain of *Candida albicans* growing as round shaped yeast cells (left) can switch to hyphal growth (right) under inducing conditions.

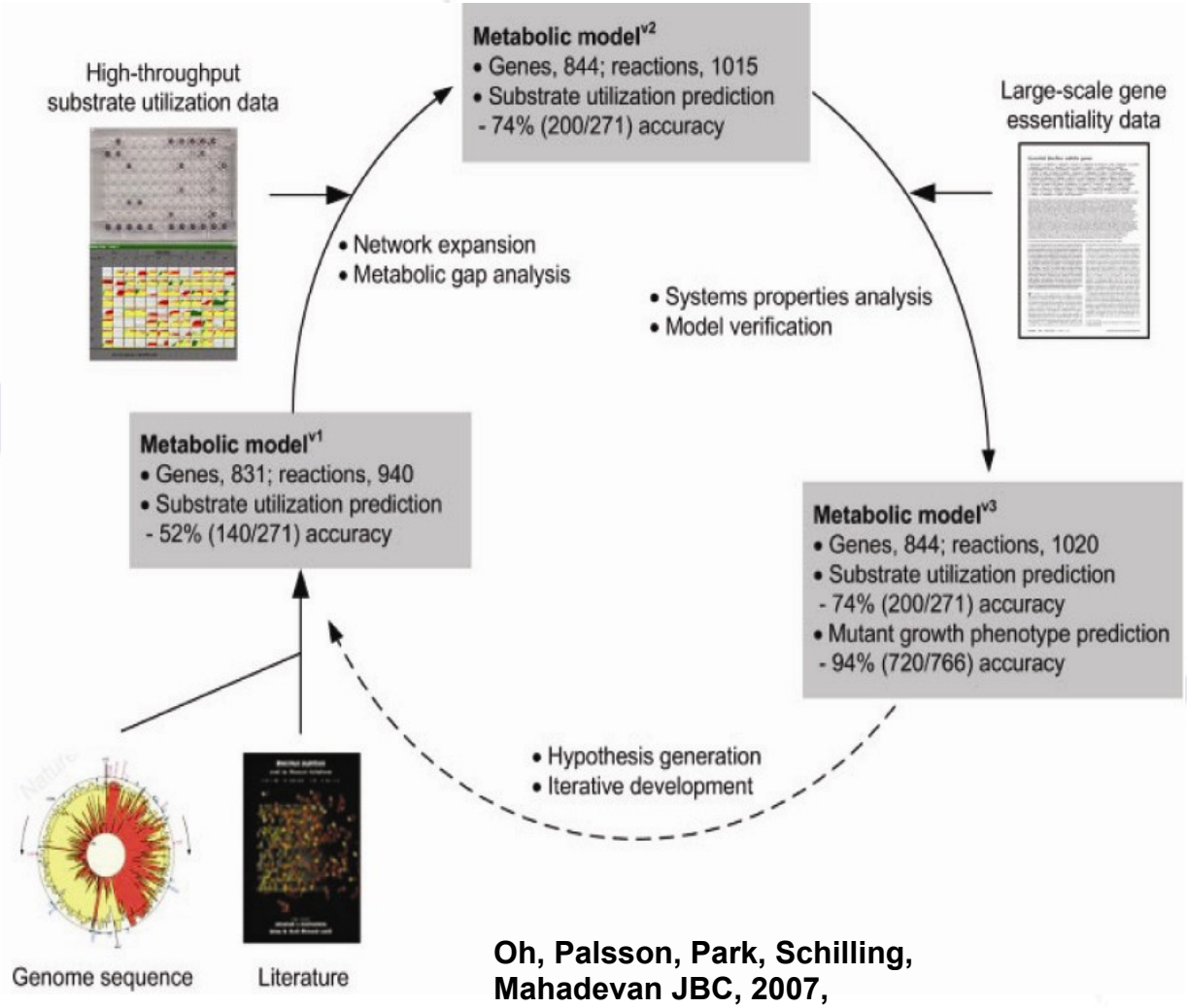
N. C. Hauser, et al., Screening (2002) 4:28-31

BIOLOG

Phenotype MicroArray Technology in Systems Biology Modeling of Cell Metabolism

BIOLOG

Using PM to Improve Annotation and Modeling



Oh, Palsson, Park, Schilling, Mahadevan JBC, 2007, 39:28791-28799

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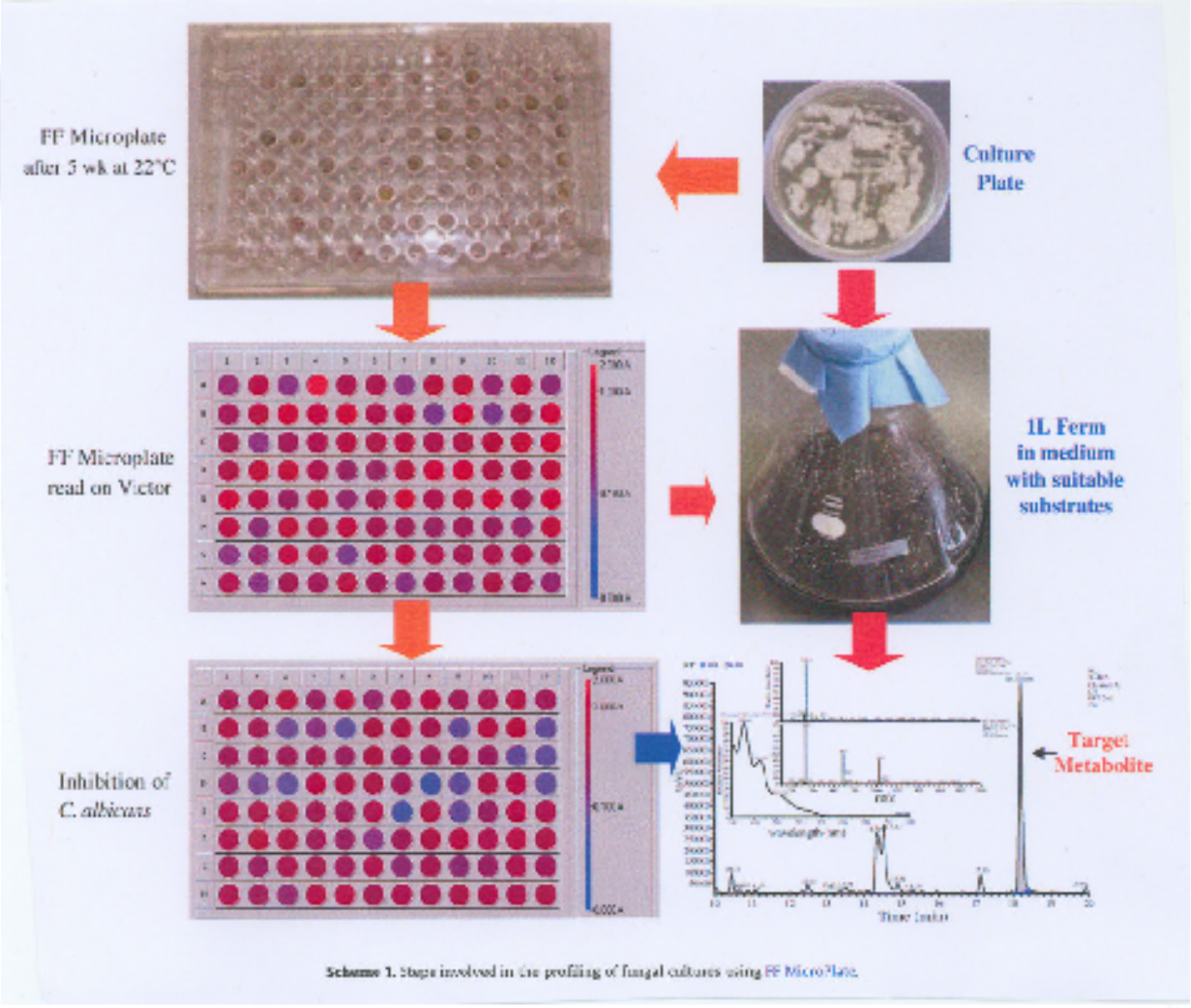
Steps in BioProcess Development Aided by PM

- Efficiently optimize many aspects of bioprocesses
- Characterize cell lines to select the best one to use
- Understand the culture properties of any cell line
- Understand how genetic changes affect the cell line
- Simulate hundreds/thousands of culture conditions: both the growth phase and production phase
- Optimize culture conditions for both rapid growth and maximum product
- Use it as a QC tool to test stock and inoculum cultures, improve process consistency, and ID contaminants

BIOLOG

Microscale Analysis of Cell Productivity - Wyeth



Scheme 1. Steps involved in the profiling of fungal cultures using FF MicroPlate.

M. Singh, J. Micro. Methods (2009) 77:102

BIOLOG

Some Major Challenges and Gaps in Cell Modeling

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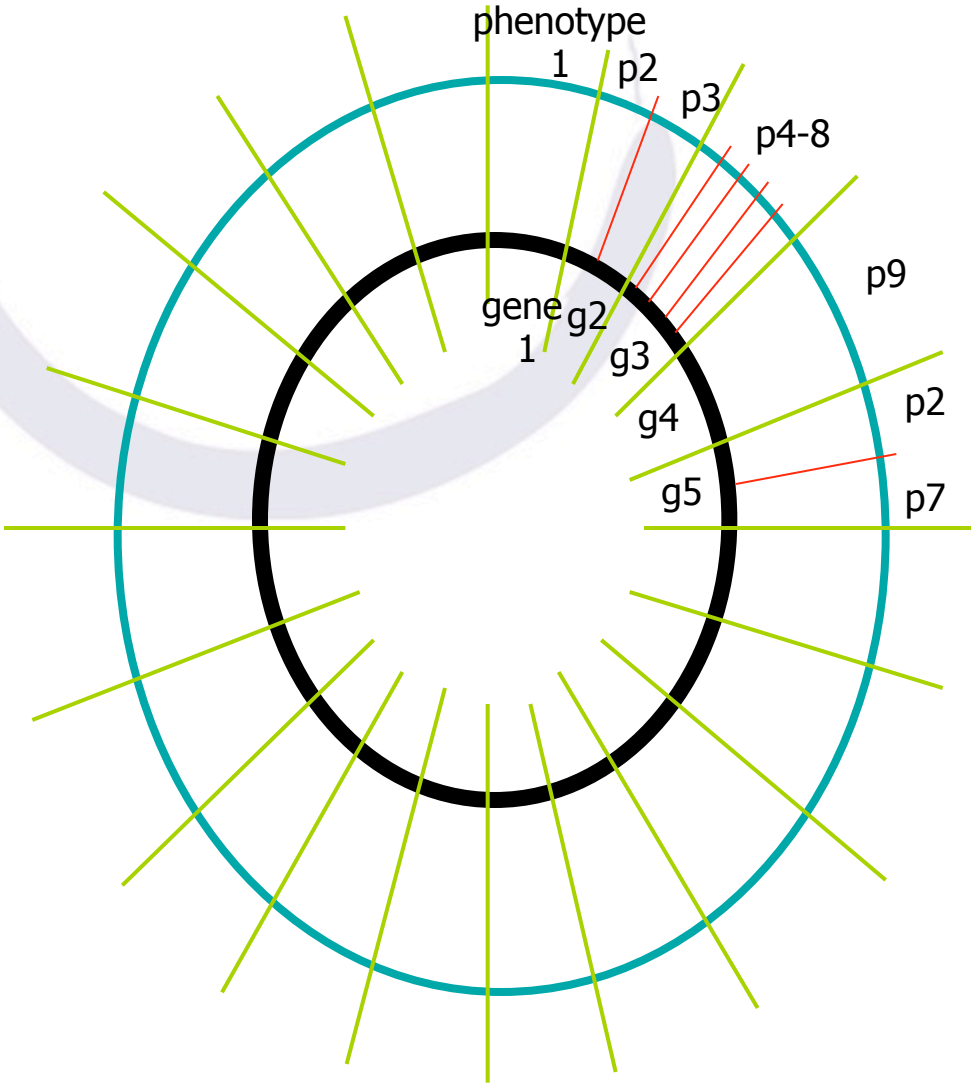
Making Phenotypic Maps



The more phenotypes that one can measure, the more completely one can describe a microorganism or mammalian cell and the more completely you can describe its genome. We need phenotypic maps to enhance genomic maps. More is better – both in quantity and variety. Ideally one would like to have a universal phenotyping set.

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Annotation of Transporter Genes in P. aeruginosa

- Ian Paulsen and coworkers (PLoS Genetics, Sept. 2008) examined phenotypes of knockouts of transporter genes and compared them with functional annotations based on DNA homology.
- Only 12/27 (44%) precisely matched predicted annotation
- In 10/27 (37%) a more precise annotation was obtained
- In 5/27 (18%) a significant reannotation was enabled
- Novel transporters were identified for L-glutamate, N-acetyl-L-glutamate, hydroxy-L-proline, and histamine

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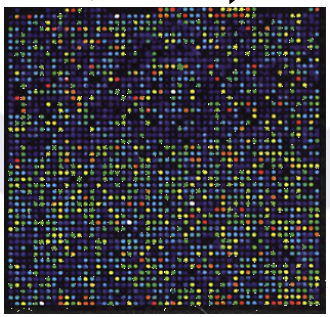
Integrating Information from OMICs Analysis

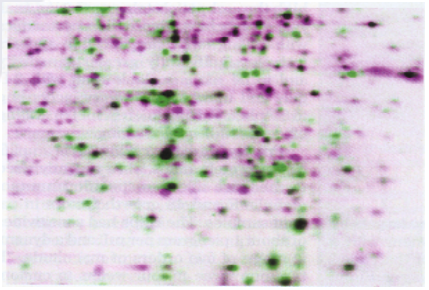
DNA → RNA → PROTEIN - - - - → PHENOTYPE

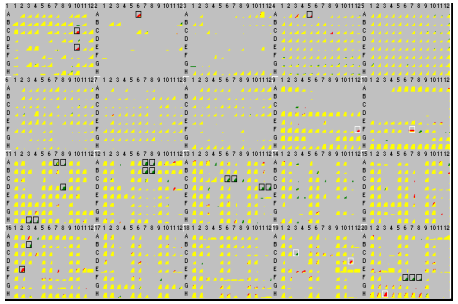
Affymetrix, 1993

O' Farrell, 1975

Biolog, 2000







Molecular Analyses

Cellular Analysis

Transcriptomics

Proteomics

Phenomics

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Addressing Other Complexities to Metabolic Regulation

- Feedback, feedforward, cross pathway regulation
- Isozyme regulation
- Global signaling with Alarmones (e.g. cAMP)
- Transcriptional regulation (E. coli has 288 trans factors)
- Regulatory RNAs (e.g. riboswitches and microRNAs)
- Modulation of transcription (e.g. histone acetylation)
- Modulation of enzyme activity (e.g. phosphorylation, acetylation, adenylation, uridylation)
- Undiscovered pathways and genes of unknown function
- Relating models to cell physiology

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What Should Our Research Priorities Be ?

genomeweb

International Team Sequences and Assembles Sea Lamprey Genome

February 25, 2013

International Team Sequences and Assembles Sea Lamprey Genome

By a GenomeWeb staff reporter

NEW YORK (GenomeWeb News) – An international team led by investigators at the Michigan State University and the University of Kentucky have sequenced and assembled the genome of sea lamprey, *Petromyzon marinus*, using the sequence to begin refining their understanding of vertebrate evolution. Their work was published online yesterday in *Nature Genetics*.

"The lamprey genome provides an important resource for reconstructing vertebrate origins and the evolutionary events that have shaped the genomes of extant organisms," senior author Weiming Li, with MSU's Department of Fisheries and Wildlife, and colleagues explained.

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Acknowledgements

- Funding from NIH (NIGMS, NIAID, NCI, NIMH)
- Also DOE and NASA and NSF
- All of my colleagues past and present at Biolog, Inc.

BIOLOG

Metabolic and Phenotypic Analysis and Identification of Microbial and Mammalian Cells

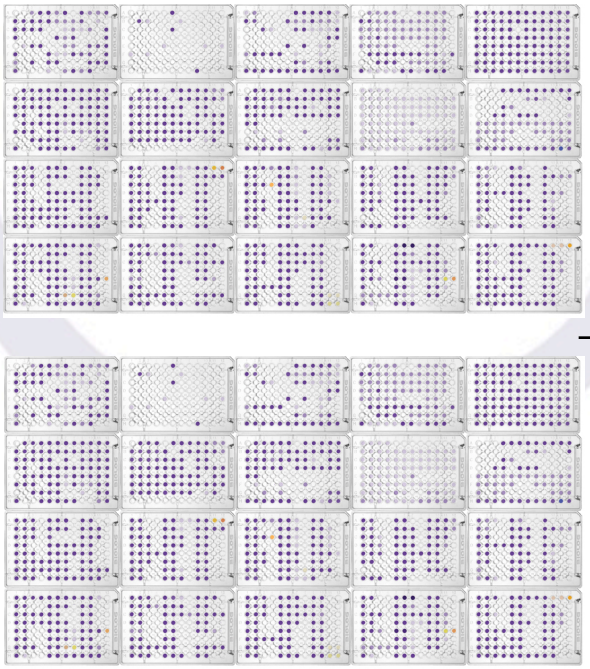
Barry Bochner, PhD

CEO & CSO

Biolog, Inc

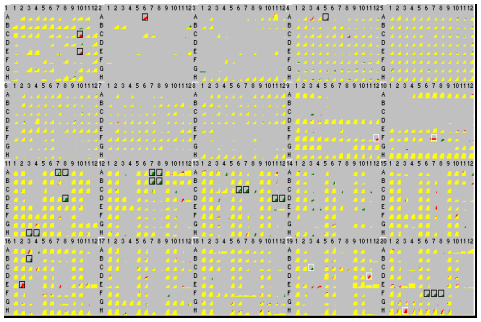
Drug Testing with PM Technology

Without Drug



With Drug
(various concentrations)

OmniLog PM System



PM Kinetic Result

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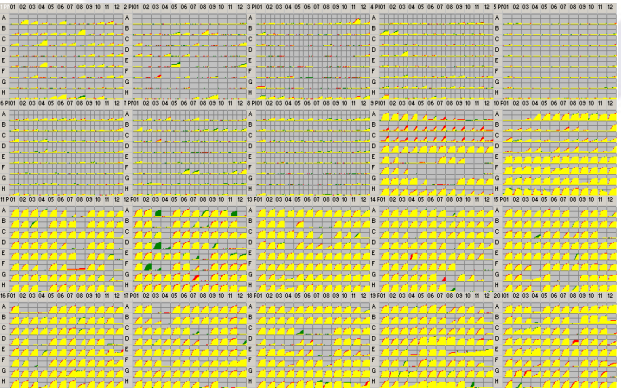
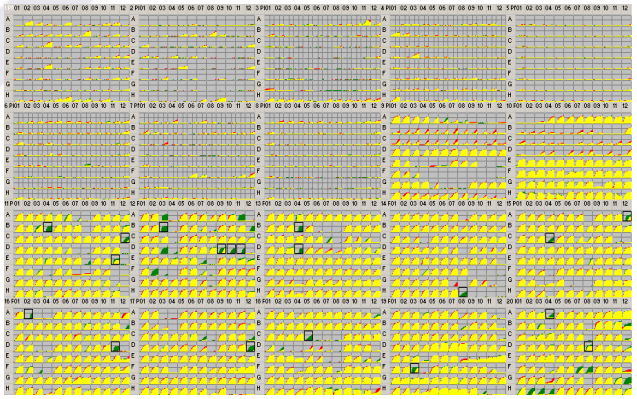
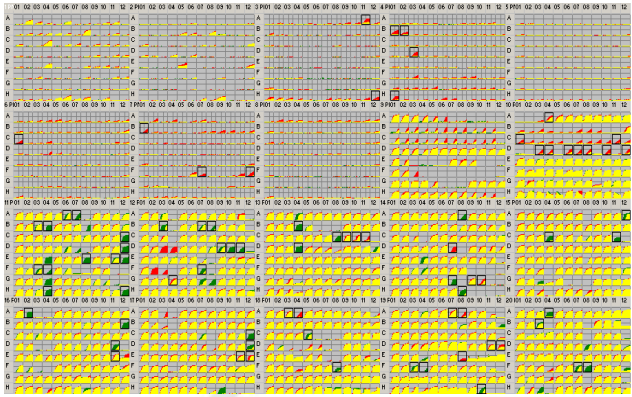
Drug vs Phenotype Titration

10µM **100µM** **1000µM**







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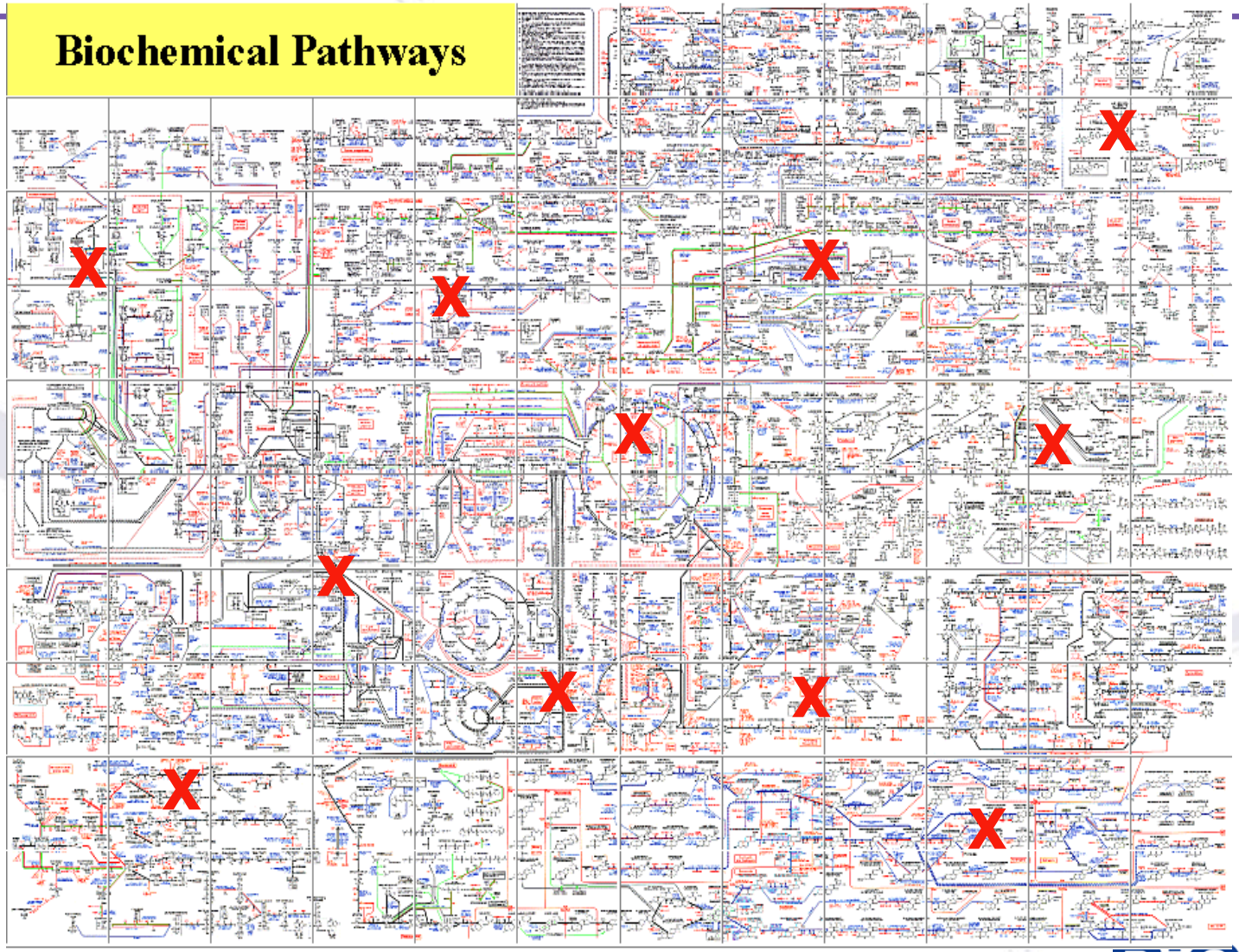
Inhibitors Knockout Various Pathways



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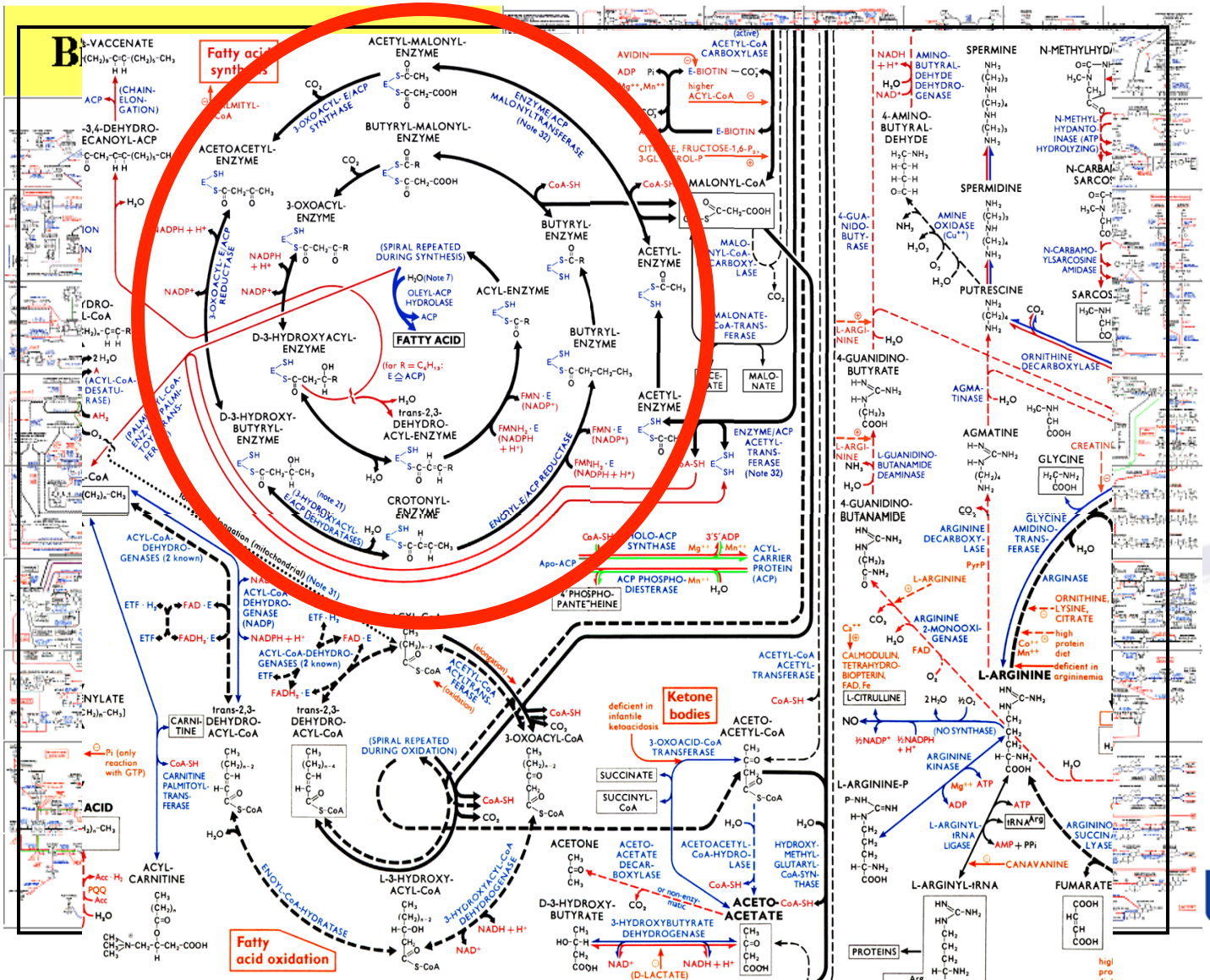
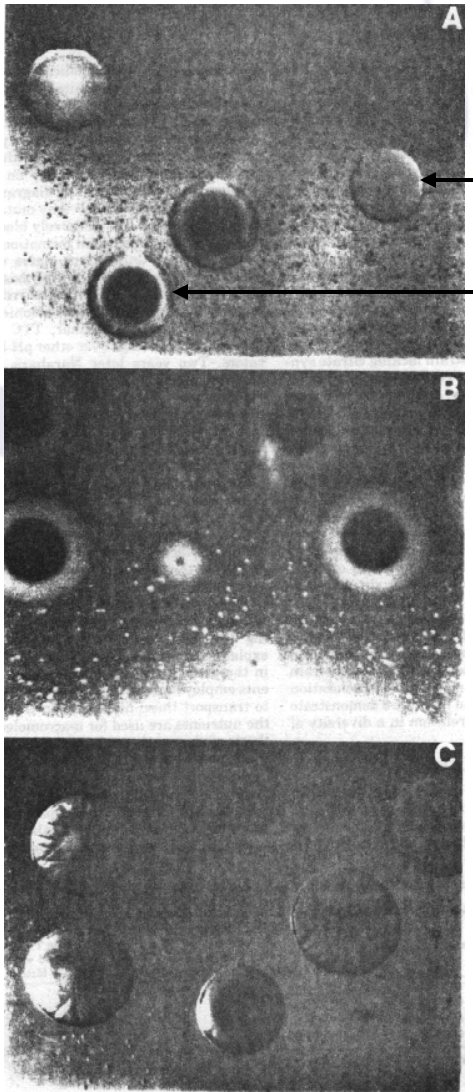
Simulating Global Metabolism



Accidental Discovery in 1975



Histidine non-metabolizing colonies (hut-) are white

Histidine metabolizing colonies (hut+) are red

This discovery became my first scientific publication, most of my PhD dissertation, most of my scientific career

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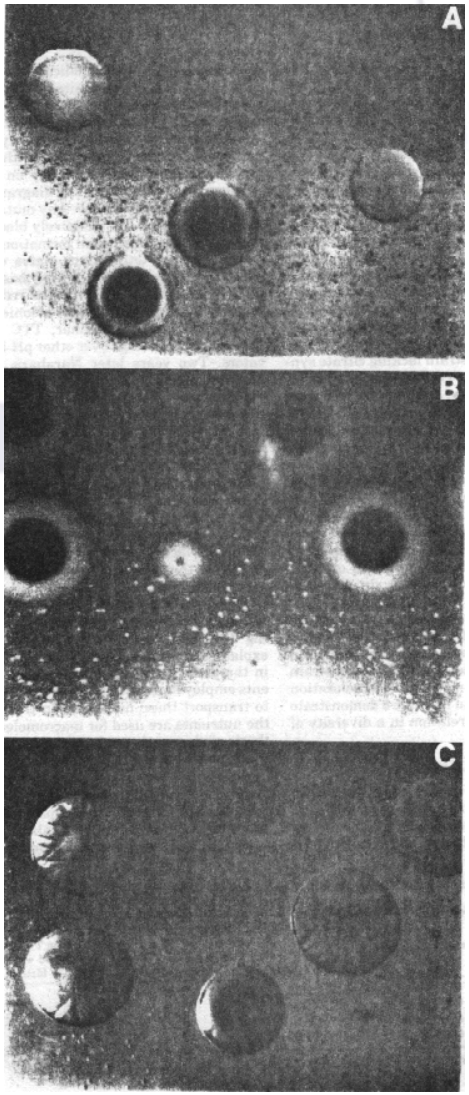
Tetrazolium Redox Dyes as Universal Indicators



Colonies with red centers indicate metabolism of the carbon source

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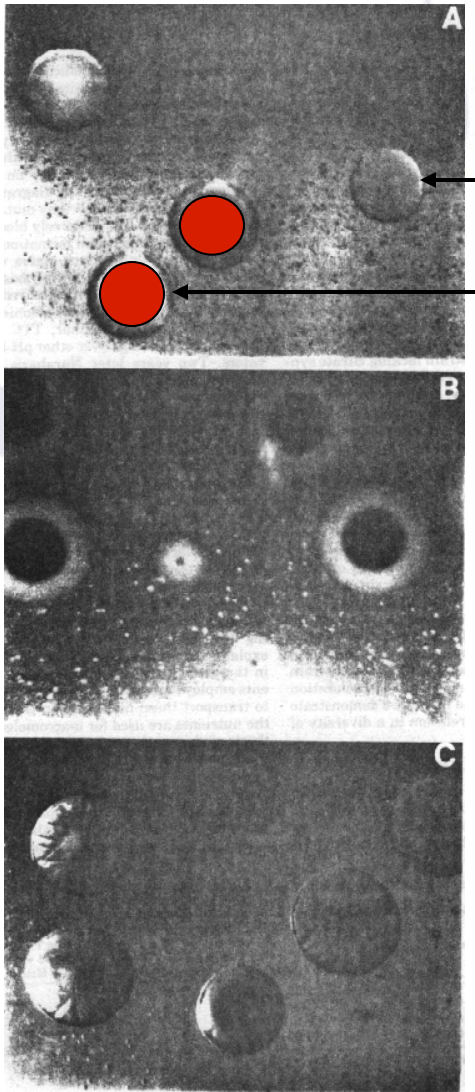
Accidental Discovery in 1975



Histidine non-metabolizing colonies (hut-) are white

Histidine metabolizing colonies (hut+) are red

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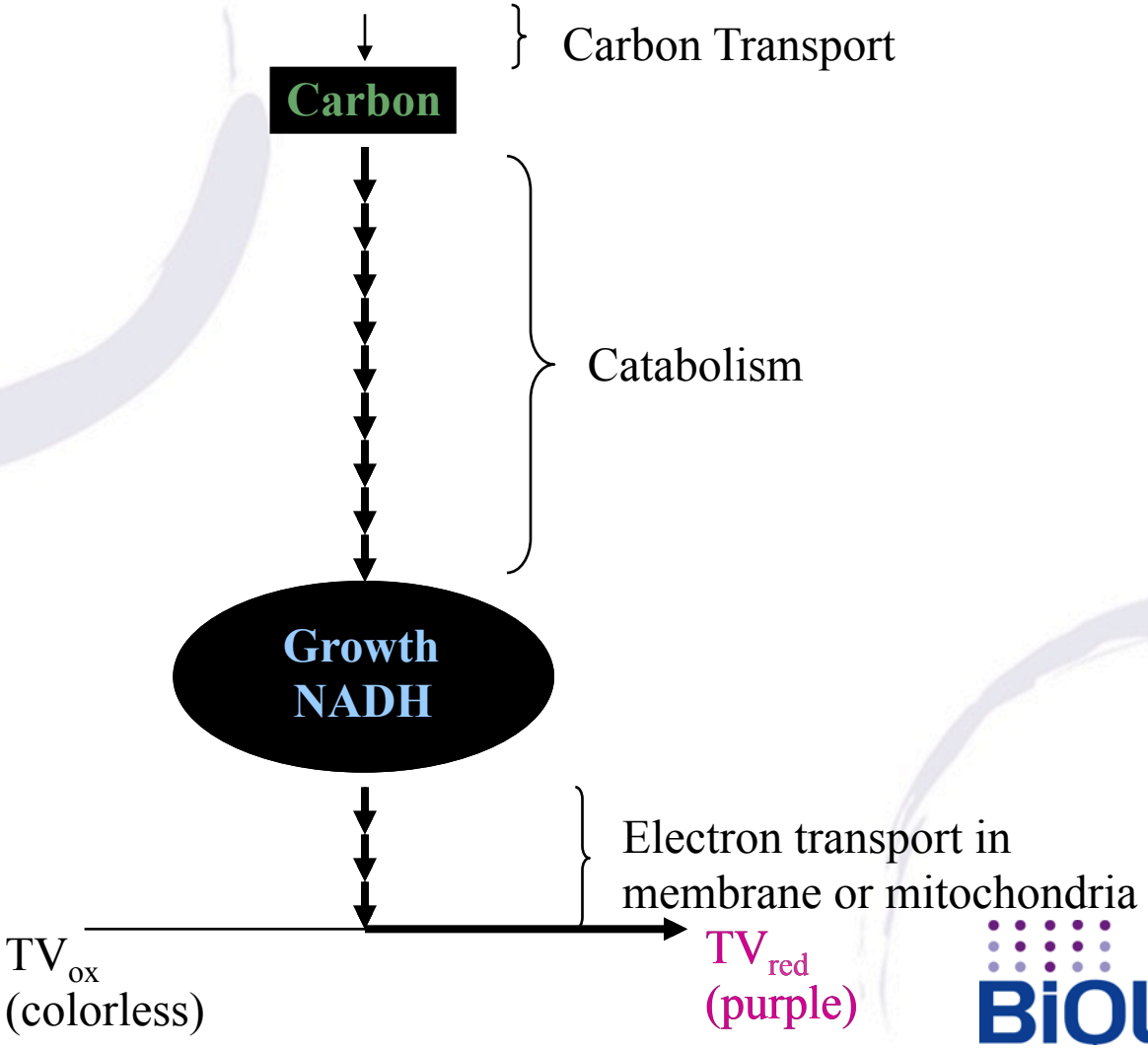
Electron Flow from C-source to Redox Dye



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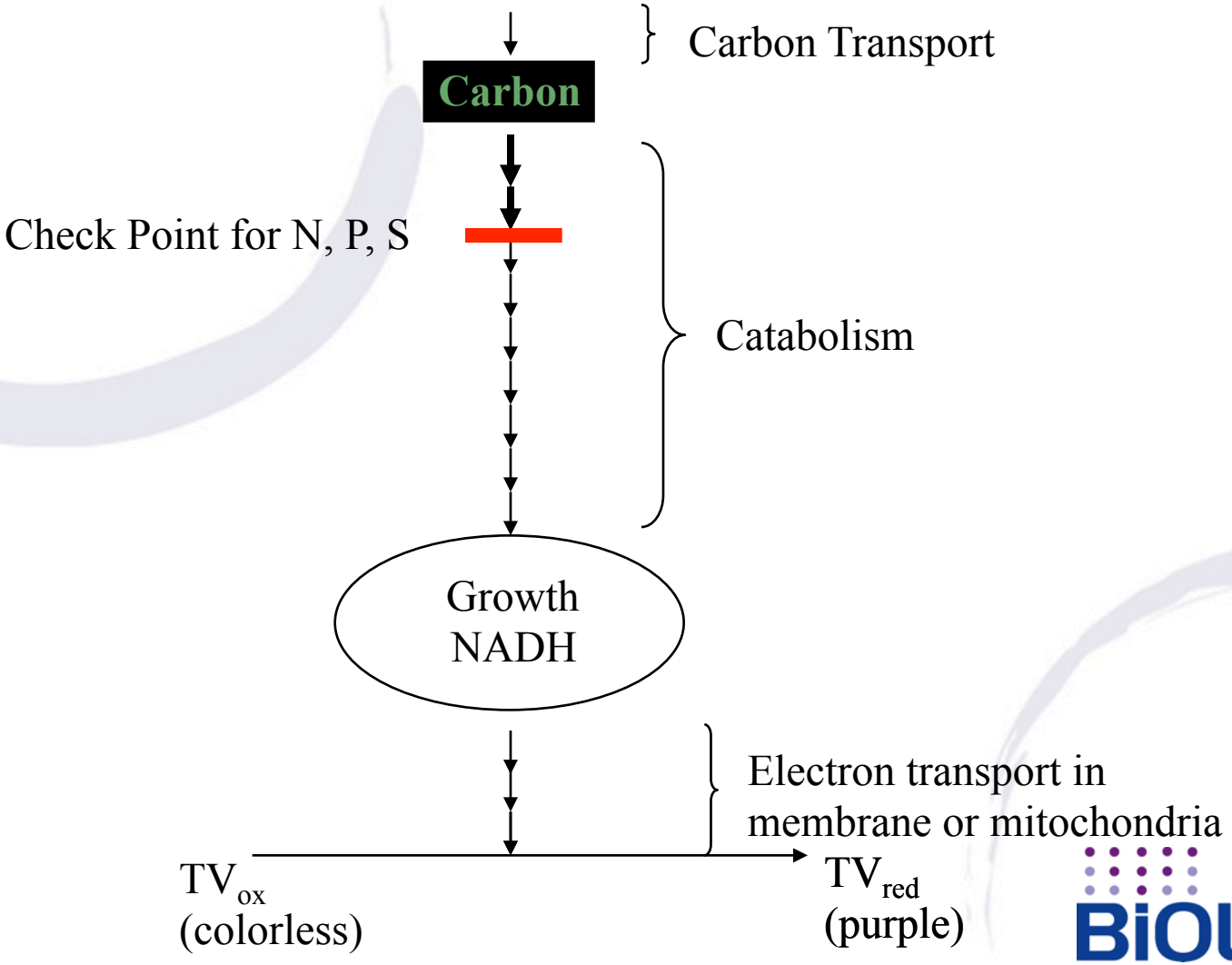
Electron Flow from C-source to Redox Dye



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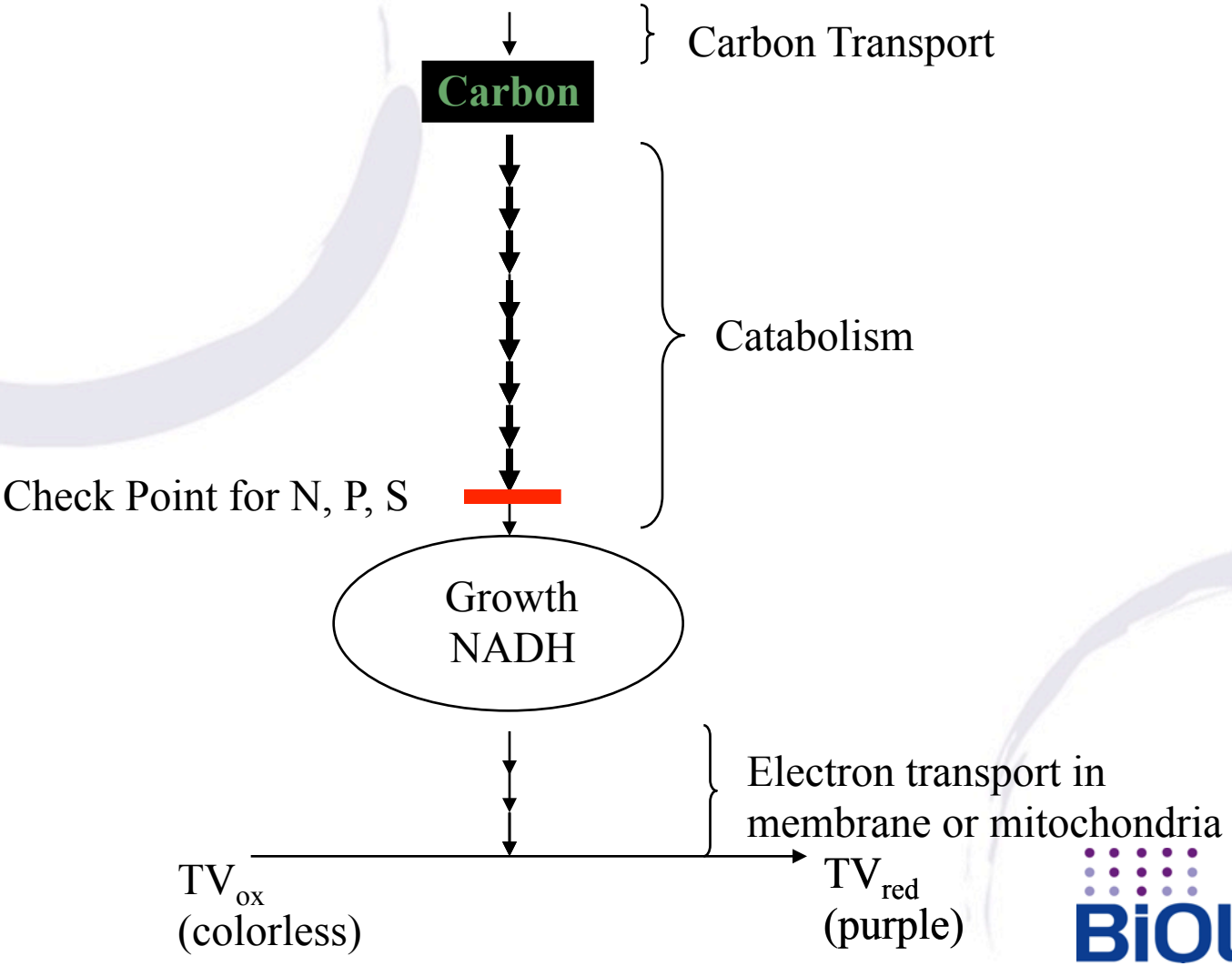
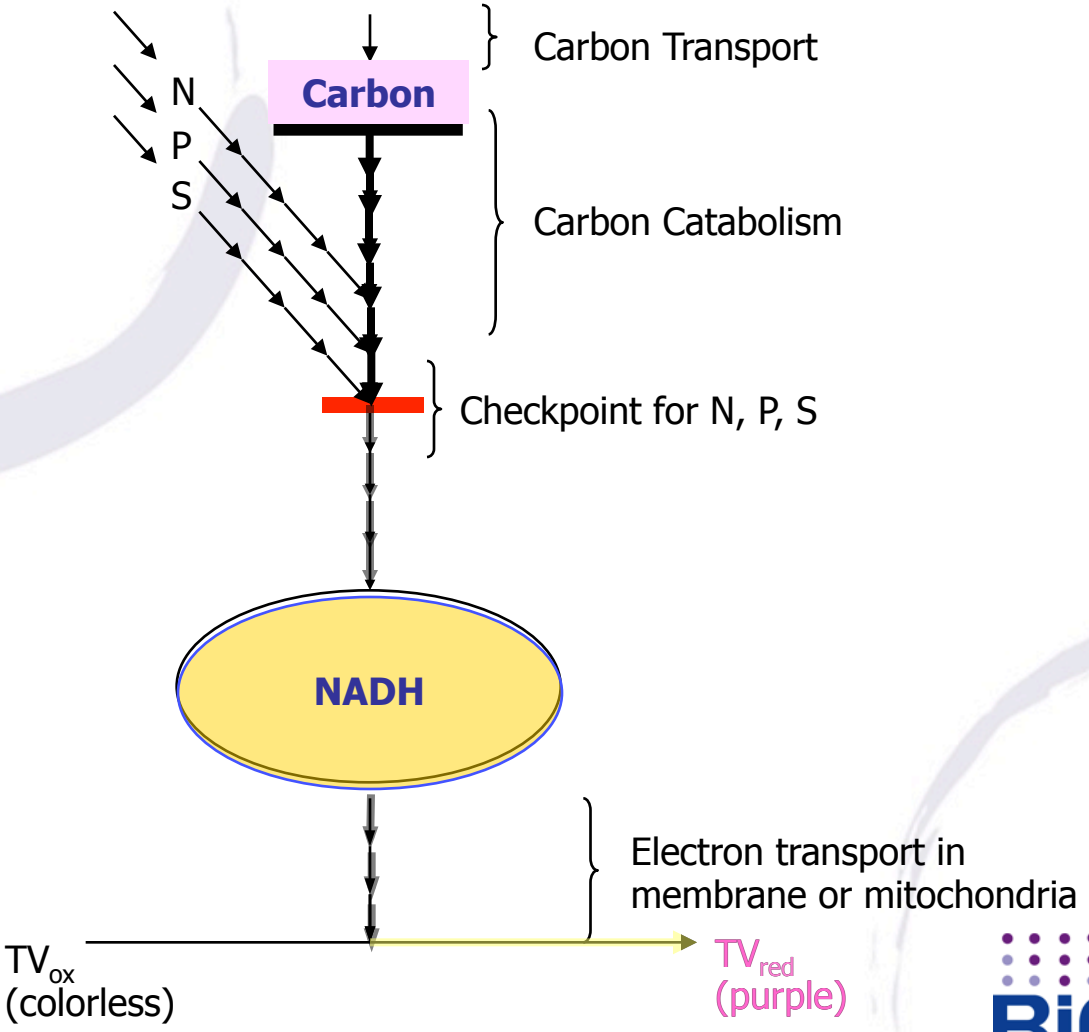
Electron Flow from C-source to Redox Dye



Electron Flow from C-source to Redox Dye



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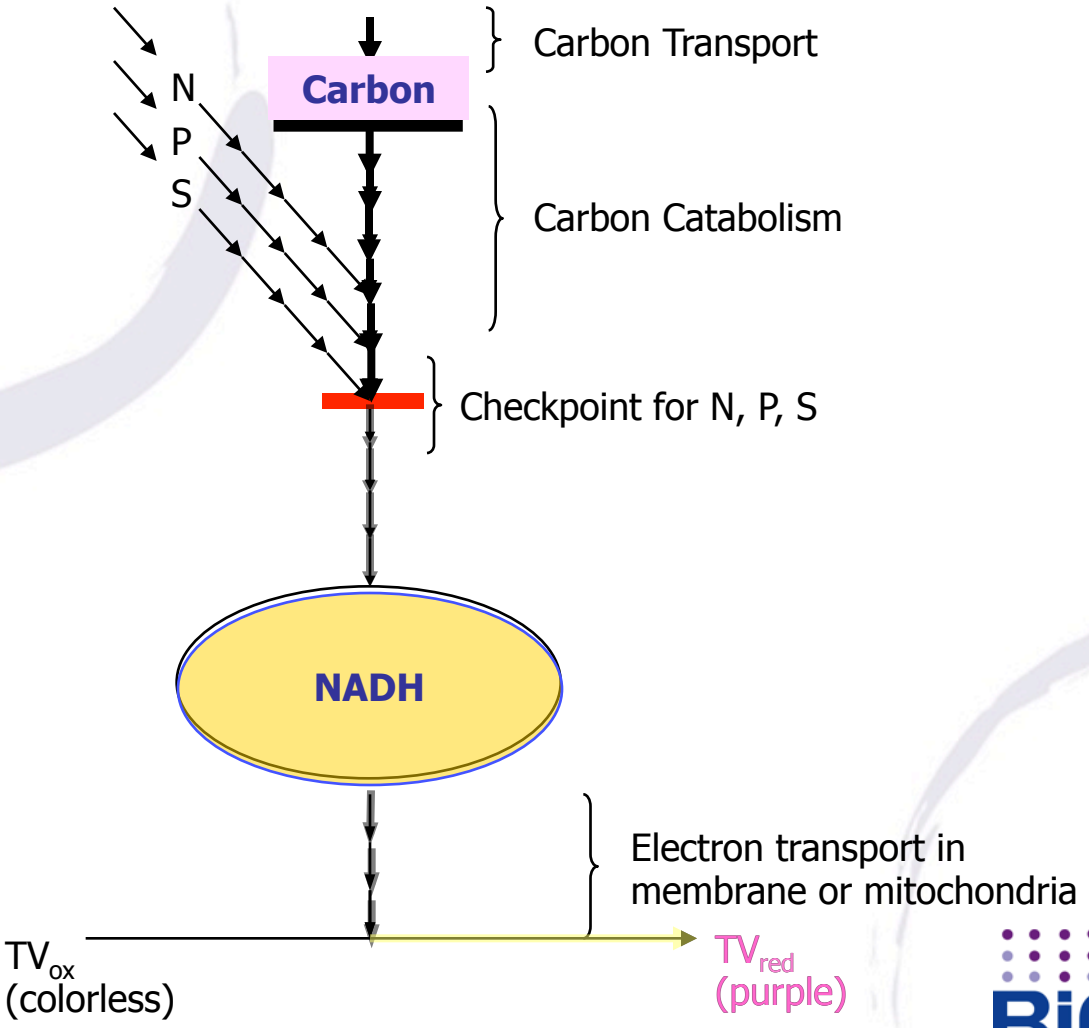
Electron Flow from C-source to Redox Dye



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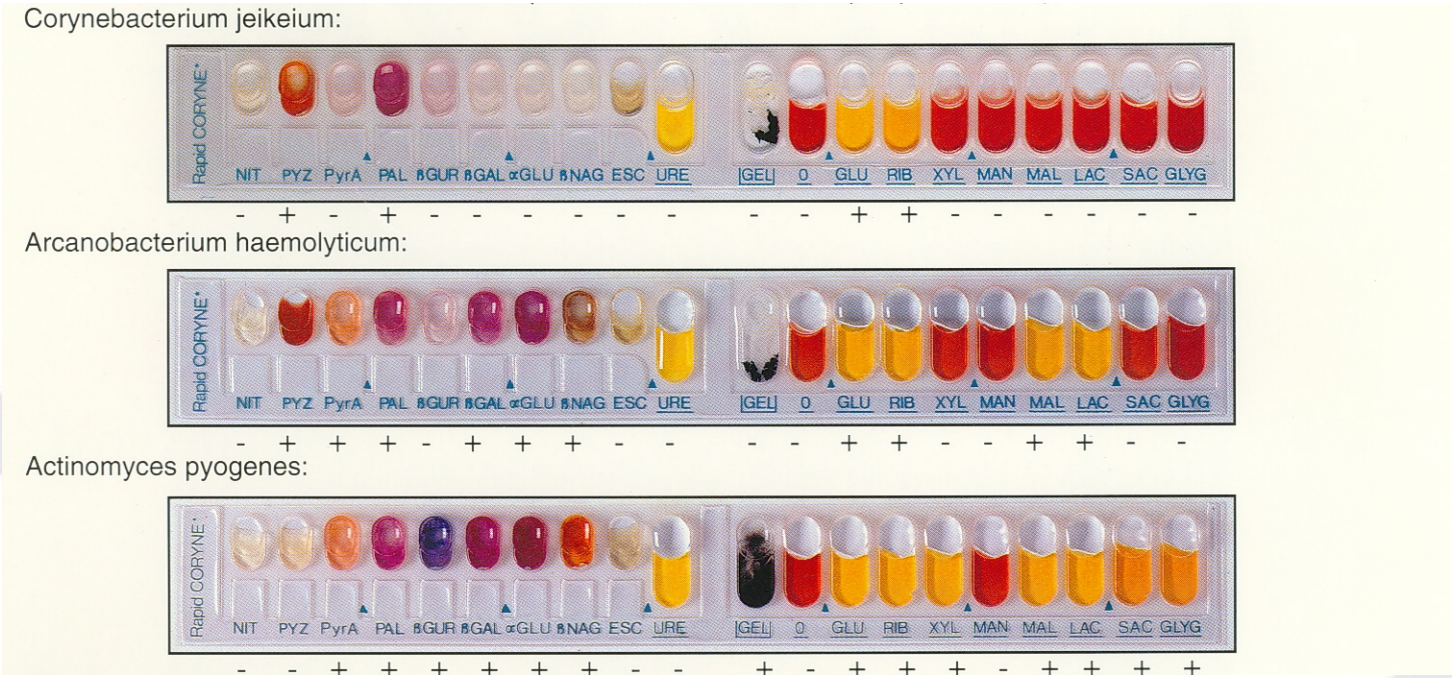
Microbiology Test Kits in the 1970s



"Clinical systems" use pH indicators (which only work well for acid-producing species) and assorted chromogenic tests (which must be invented and developed one-at-a-time)

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Characterization of Fermentation Strains

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PM Analysis of Streptomyces coelicolor

succinate mannitol glutamate gelatin
glycerol tweens lactose gentiobiose

nitrite, urea
most amino acids (not met)

prototrophic

no met peptides

osmotically sensitive except to urea

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From a Redox Color Change to Scanning Cell Physiology

From Scanning Cell Physiology to Important Discoveries

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