

Protein Expression Systems

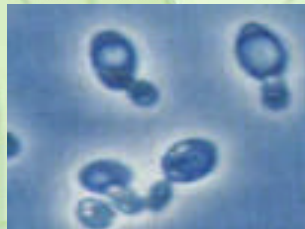
Do we really need another one...?

Bacteria



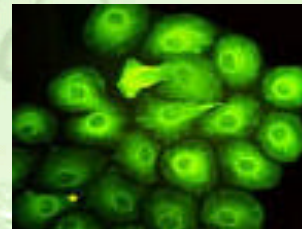
- Insufficient folding of complex proteins of higher organisms
- Lack of post-translational modifications
- Endotoxins

Yeast



- Posttranslational modifications differs largely from mammalian cells
- Problematic cell disruption

Insect & Mammalian Cells



- Laborious construction of over-expressing strains
- Expensive media
- Low growth rates
- Difficult scale-up
- Low expression levels

Transgenic Plants & Animals

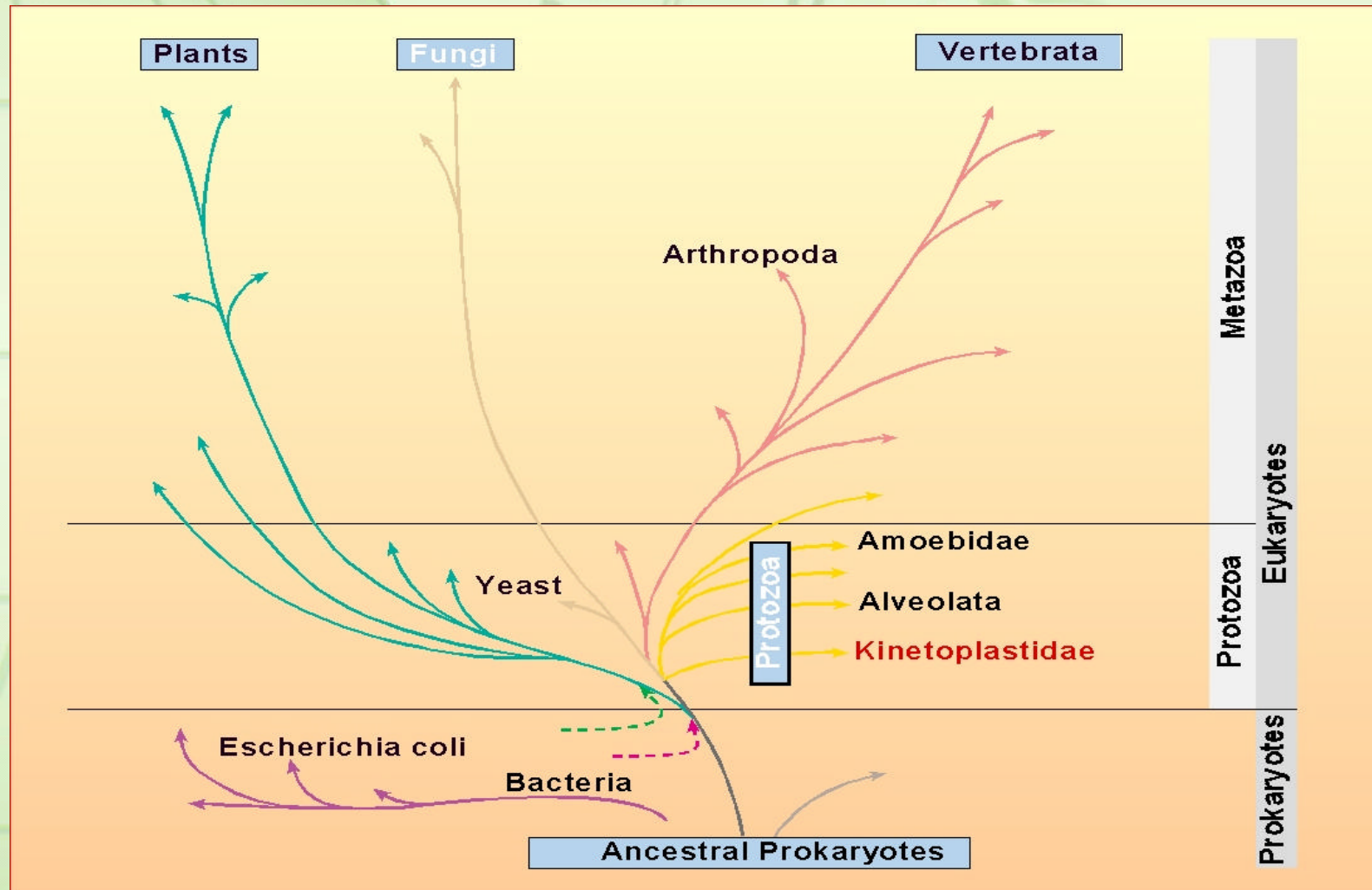


- Long developmental cycles
- Complex downstream processing
- Contamination problems

Current expression systems not ready for genomics era
Demand for eukaryotic machinery but *E. coli*-like properties and handling

Leishmania – a closer relative than one may think

Full eukaryotic protein folding and modification machinery



Jena Bioscience *Leishmania tarentolae* Expression System

Is now marketed as LEXSY

► Rapid growth

- Cultivation in cost-efficient media at 26°C, doubling time 4 h⁽¹⁾
 - Serum-free standard media or fully synthetic media
- Cell densities in suspension cultures >10⁸ cells/ml

► Non-pathogenic to humans, lizard-parasite

- Received biosafety S1-clearance

► Full eukaryotic protein folding machinery

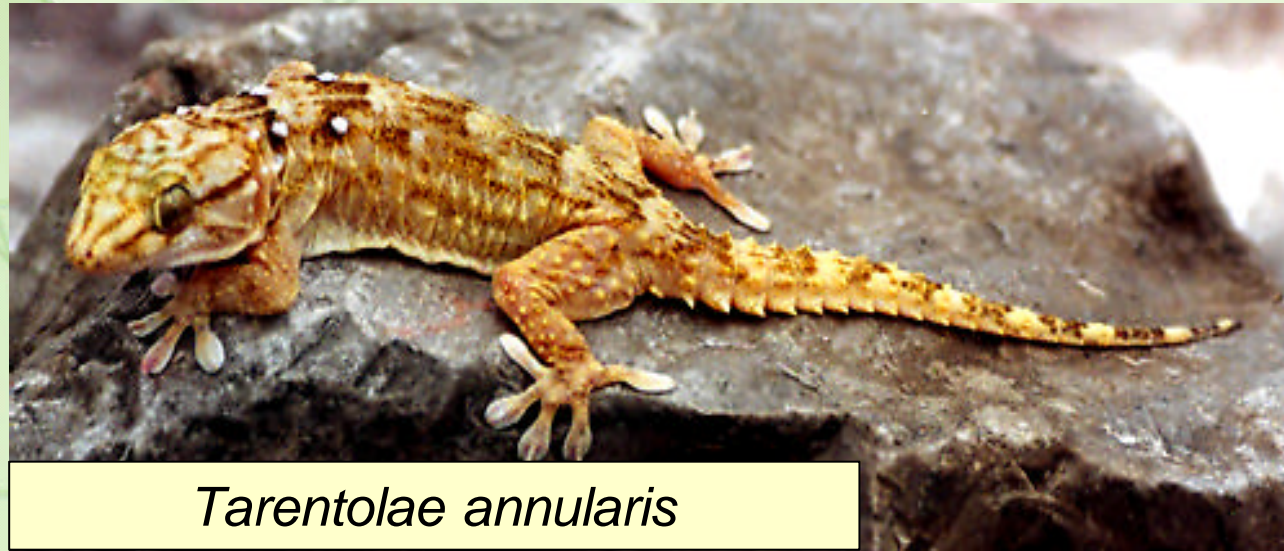
► Mammalian-type posttranslational protein modifications

Patent held by Jena Bioscience GmbH (WO 01/32896 A1)

(1) In agitated cultures, approx. 7 h in static cultures

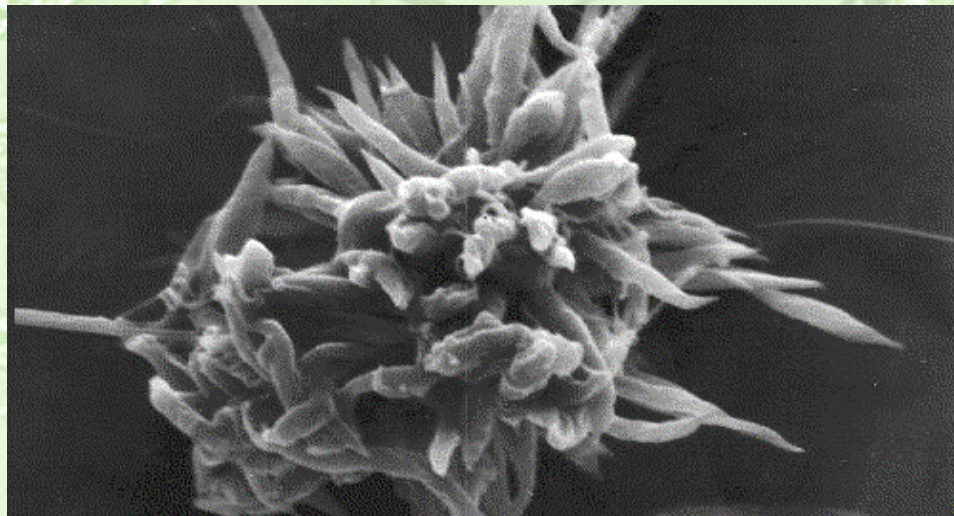
backup

Host



Tarentolae annularis

Parasite

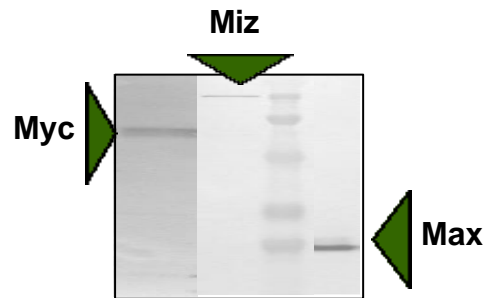


Leishmania tarentolae

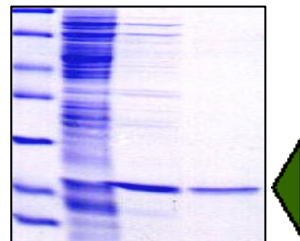
Protein Expression: The Power of LEXSY

Expression of Selected Proteins of Interest at Jena Bioscience

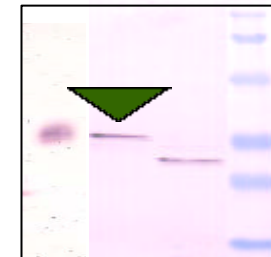
Active human transcription factors produced in LEXSY only



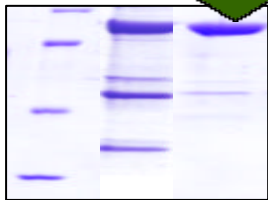
Native human SOD purified to homogeneity



Human recombinant EPO homogenously glycosylated

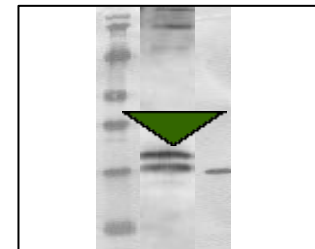


Superior expression of huPI3 kinase accessory protein p85 in LEXSY compared to Sf21

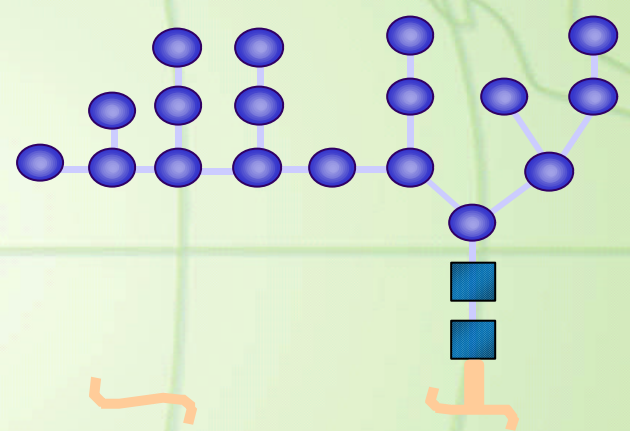


Leishmania tarentolae

Human Interferon Gamma is expressed in two molecular forms as known from human preparations

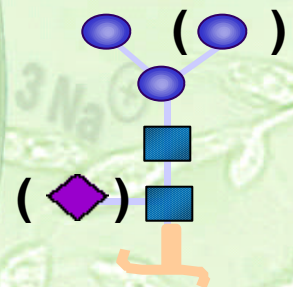


LEXSY N-Glycosylation Pattern Similar to Mammals

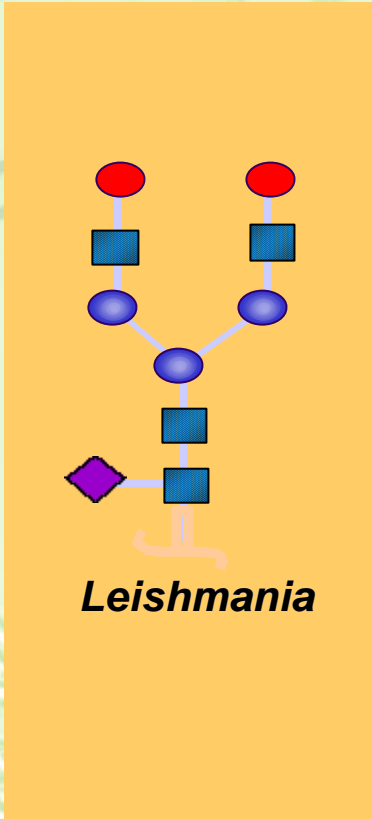


Bacteria

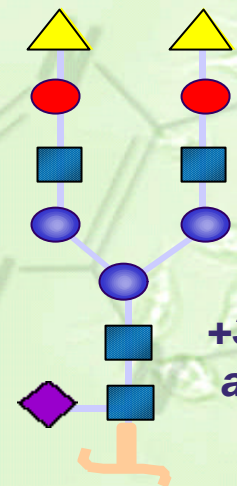
*Yeast
e.g. Pichia*



*Insect cells
e.g. Sf9/21*



Leishmania



*Mammalian
cells*

	Galactose		N-acetylneuraminic acid
	Mannose		N-acetylglucosamine
	Fucose		Polypeptide

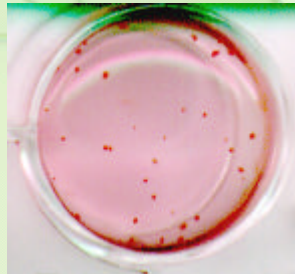
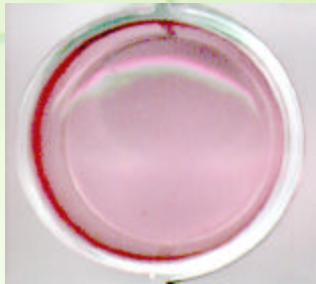
Activity of rhu EPO from LEXSY competes well with CHO-EPO

CFU-GEMM* cell proliferation assay
hu CD34⁺ peripheral blood stem cells

Dose-dependent proliferation and
differentiation in haemoglobin
producing cells

NK

PK

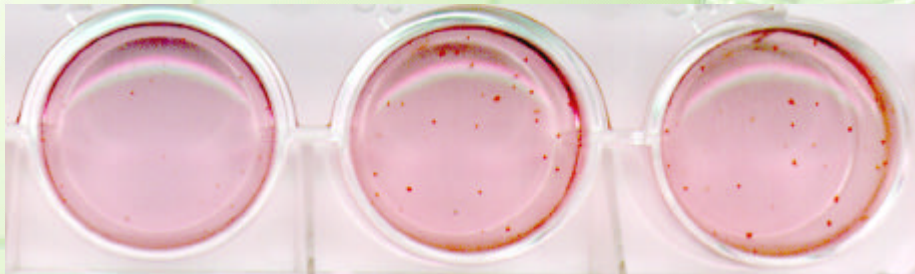


5 U EPO
(CHO)

1 U

5 U

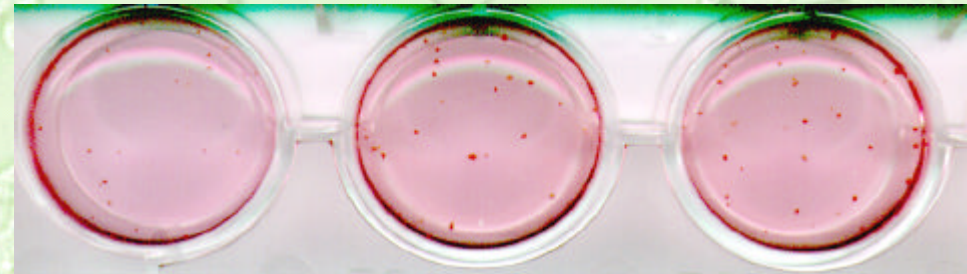
10 U



0.65 U

1.3 U

3.6 U



Native signal peptide

1.2×10^5 U/mg

Leishmania signal peptide

4.0×10^5 U/mg

* Granulocytes-erythrocytes-monocytes-macrophages