Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute

Epithelial Invasion Outcompetes Hypha Development During *Candida albicans* Infection as Revealed by an Image-based Systems Biology Approach

Leibniz Association

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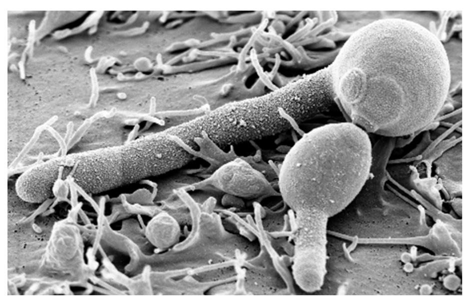
– FungiNet –

Candida albicans interaction with epithelial cells

C. albicans on epithelial cells:



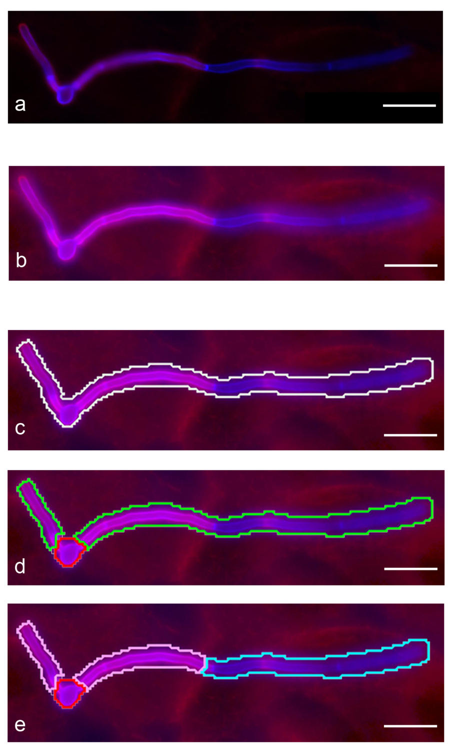
Naglik *et al.* (2011), Microbes Infect.

Candida albicans is the most common opportunistic fungal pathogen on human mucosal surfaces. Infections of the polymorphic fungus *C. albicans* begin with adherence of yeast cells to host tissue which is followed by epithelial invasion. This process is enforced by either the fungal hyphae (active penetration) or the host (induced endocytosis). The switch from yeast to hyphal forms is an important virulence attribute. Applying an Image-based Systems Biology approach we are able to elucidate the complex morphological kinetics during *C. albicans* epithelial interactions [1].

[1] Mech *et al.* (2014), *Cytometry Part A* 85(2)

Automated Image Analysis

Scheme of image analysis using *Definiens*®



(a) The original image contains two layers, one for blue and one for red fluorescent label. Scale bars correspond to $10\mu m$

Pre-processing:

(b) Image after maximum intensity transformation from $z-stacks$ into 2D-images.

Segmentation & Classification:

(c) Segmentation of the fungal cell as region of interest (ROI) by intensity thresholding.

(d) Classification of the yeast cell (red outline) and its hyphae (green outline) by morphological differences.

(e) Classification of invaded (blue) and non-invaded (pink) segments by differential staining.

Readout from image time-series of 6 h:

Labeled classes
- yeast cell
- filamentous cell
- hypha
- invaded segment
- non-invaded segment

Quantitative measures
- number of hyphae per filamentous cell
- hyphal segment length

Is invasion preferred over hyphae generation?
▷ **Kinetic Transition Model**

What are the growth rates of invaded and non-invaded hyphae?
▷ **Kinetic Growth Model**

Mathematical Models and Parameter Estimation

Kinetic Growth Model

Input from image analysis:
- kinetics of hyphal growth
- distinction between invaded and non-invaded length per fungal cell

Ordinary Differential Equation (ODE) Model

$$L(t) = L_{ni}(t) + L_i(t)$$

$$\frac{dL_{ni}(t)}{dt} = \alpha_{ni} F_{ni}(t) \qquad \frac{dF_i(t)}{dt} = r_i F_{ni}(t)$$

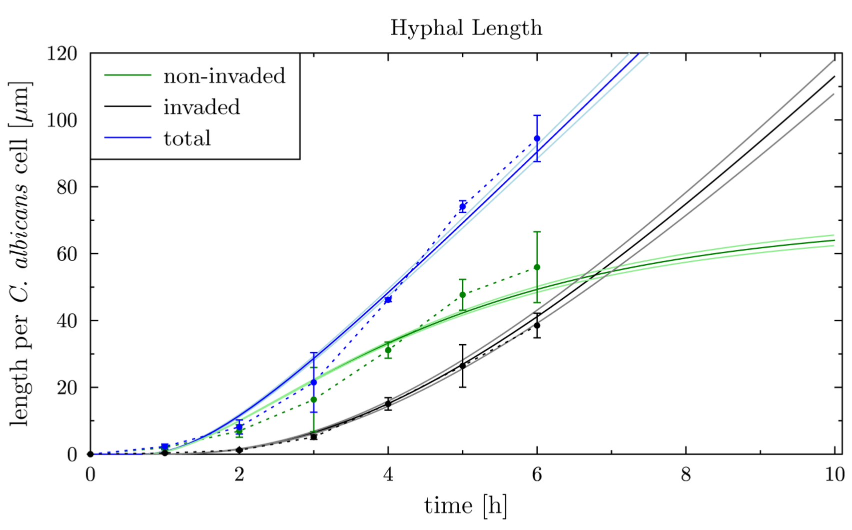
$$\frac{dL_i(t)}{dt} = \alpha_i F_i(t) \qquad \frac{dF_{ni}(t)}{dt} = rY(t) - r_i F_{ni}(t)$$

$L_{ni}(t)$ kinetics of non-invaded hyphal length
$L_i(t)$ kinetics of invaded hyphal length
α_{ni} growth rate constant of non-invasive hyphae
α_i growth rate constant for invasive hyphae
$F_{ni}(t)$ non-invasive filamentous cells
$F_i(t)$ invasive filamentous cells
r_i invasion rate of non-invasive filamentous cells
r rate of germ tube generation from yeast cells
$Y(t)$ yeast cells

Resulting rate values:

parameter	value	sample standard deviation
α_{ni}	0.346 μm/min	0.005 μm/min
α_i	0.37 μm/min	0.02 μm/min
r	0.0159 min^{-1}	0.0005 min^{-1}
r_i	0.0049 min^{-1}	0.0001 min^{-1}
t_0	42.3 min	1.1 min

Resulting hyphal length dynamics



- growth rate of invasive and non-invasive hyphae are similar: $\alpha_{ni} \approx \alpha_i$
  → increase of invasive hyphal length is caused by massive epithelial invasion with rate r_i.

Parameter Estimation

Simulated Annealing based on Metropolis Monte Carlo

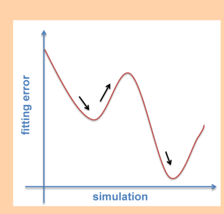
1. Choose random start parameter $\vec{p}$, calculate wLSE $\mathcal{E}_j[\vec{p}]$ for each DE j and $E(\vec{p})$

$$\mathcal{E}_j[\vec{p}] = \frac{1}{2}\sum_i (x_{i,j}^{dat} - x_{i,j}^{sim}[\vec{p}])^2$$
$$E[\vec{p}] = \sum_j \epsilon_j \cdot \mathcal{E}_j[\vec{p}]$$

2. Calculate new random parameter $\vec{p}'$ via $c-$percentage variation of $\vec{p}$ with $\mathcal{E}_j[\vec{p}']$ and $E[\vec{p}']$

$$\vec{p}' = \vec{p} \pm c \cdot \vec{p}$$

3. Compare $\vec{p}$ and $\vec{p}'$ via ΔE



→ always accept parameter values that improve fitting
→ accept parameter values that impair fitting based on Boltzmann distribution

$\Delta E = E[\vec{p}'] - E[\vec{p}]$

if $\Delta E \leq 0$ then $\vec{p} \leftarrow \vec{p}'$
else *Metropolis step:*
if $\exp^{(-\tau(f) * \Delta E)} > r$
then $\vec{p} \leftarrow \vec{p}'$
$r =$ uniform random number $\in [0, 1]$

4. Go to 2. until number of fitting steps is reached

Kinetic Transition Model

Input from image analysis:
- number of hyphae per filamentous cell
- invaded and non-invaded segments

Master Equation Model

$$\frac{\partial P(s,t)}{\partial t} = \sum_{s'} \{P(s',t) r(s' \to s) - P(s,t) r(s \to s')\} + P(na,t) r(na \to 0)\{\delta_{s,0} - \delta_{s,na}\}$$

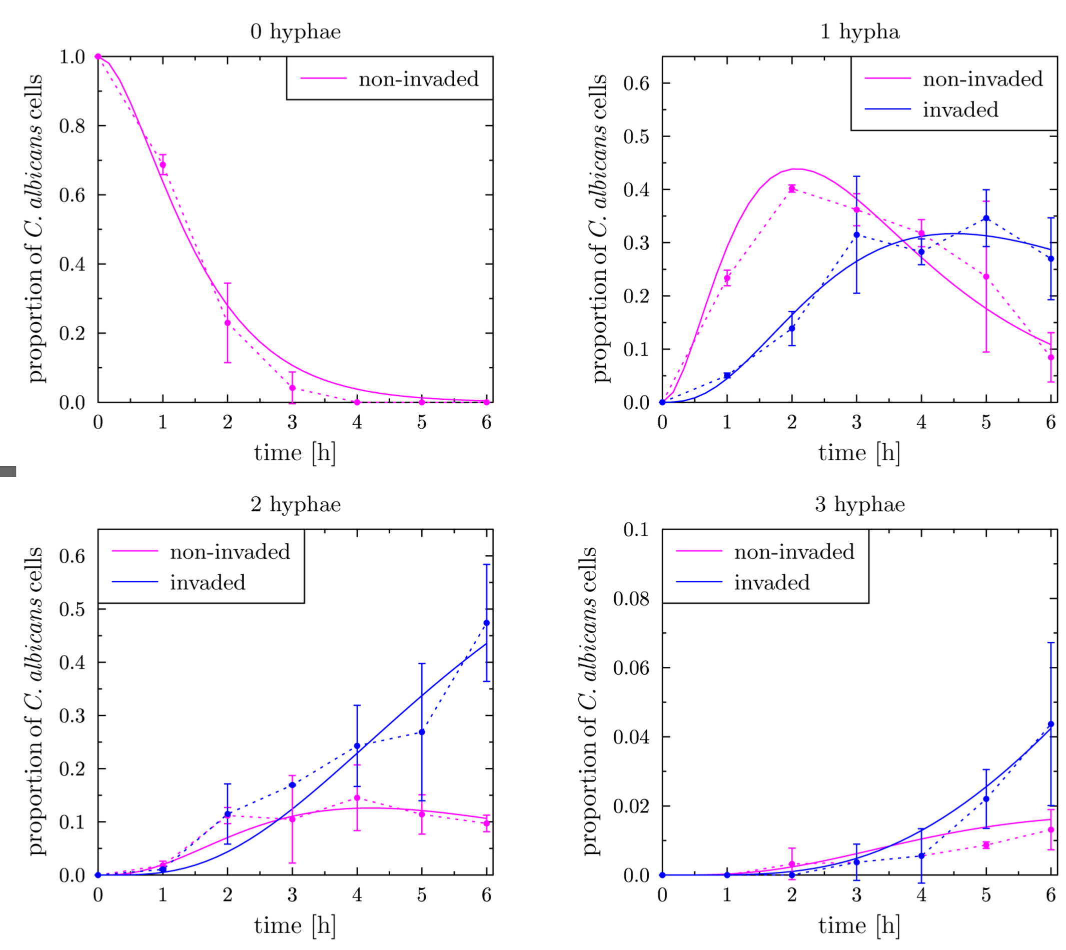
$P(s,t)$ probability to find *C. albicans* cell in a certain state s at time t
$r(s \to s')$ rate to change from state s to state s'

$$\delta_{i,j} = \begin{cases} 1 & \text{if } i = j \\ 0 & \text{else} \end{cases}$$

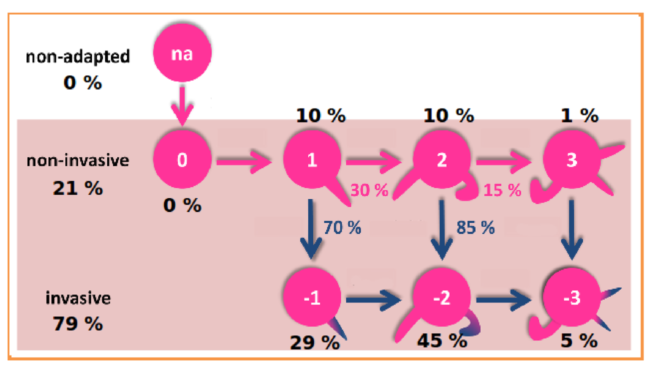
Resulting transition rate values:

	rate constant	sample standard deviation
$r(na \to 0)$	0.0211 min^{-1}	0.0007 min^{-1}
$r(0 \to 1)$	0.0212 min^{-1}	0.0007 min^{-1}
$r(1 \to 2)$	0.0028 min^{-1}	0.0006 min^{-1}
$r(1 \to -1)$	0.0063 min^{-1}	0.0006 min^{-1}
$r(-1 \to -2)$	0.0044 min^{-1}	0.0009 min^{-1}
$r(2 \to 3)$	0.0009 min^{-1}	0.0004 min^{-1}
$r(2 \to -2)$	0.005 min^{-1}	0.002 min^{-1}
$r(-2 \to -3)$	0.0006 min^{-1}	0.0001 min^{-1}
$r(3 \to -3)$	0.004 min^{-1}	0.004 min^{-1}

Resulting hyphal development dynamics



Hyphae development and invasion process after 6 h



- all yeasts form hyphae within 6 h
- most filamentous cells are invasive (79%)
- invasion is more probable than development of further hyphae (see transitions $(s \to -s)$)

Conclusion

- quantification of infection processes by an image-based systems biology approach
- initiation of hyphae formation is directly followed by invasion outcompeting formation of further hyphae
- yeast to hypha transition must be under exquisitely tight negative regulation to avoid the transition from commensal to pathogen invading the epithelium

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