Molecular Genetics and Genomics

Genetic interactions between a phospholipase A_2 and the Rim101 pathway components in *S. cerevisiae* reveal a role for this pathway in response to changes in membrane composition and shape

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Supplemental Material

Supplementary methods

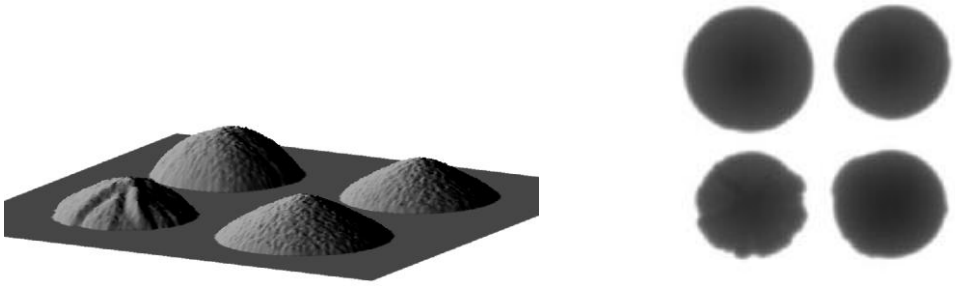
Plate scanning procedure and software used for image analysis

Plates were scanned using Epson Perfection V700 Photo scanner with transmission scan settings and 600 dpi resolution. TIFF images (inverted greyscale 16 bit files) of plates were analyzed using GenePix Pro 5.1 software (Axon).

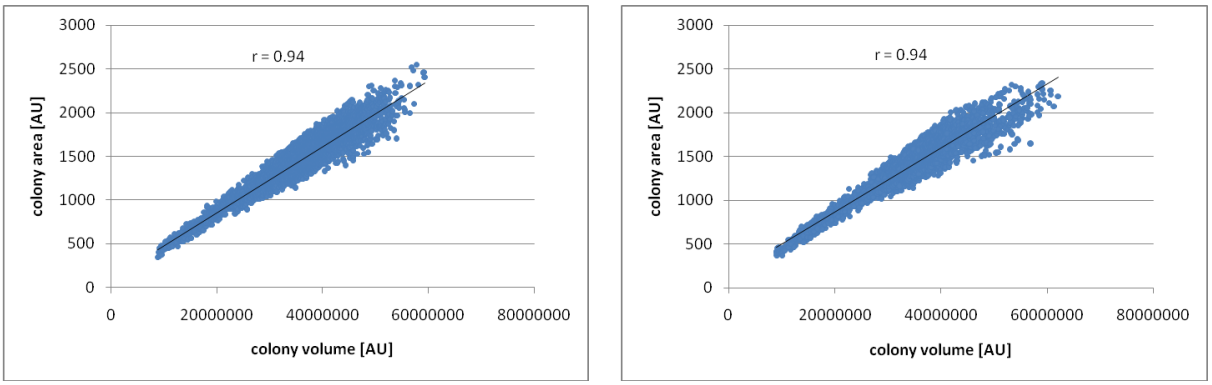
Correlation between detected colony intensities and colony volume

The basic premise of our plate analysis protocol is that due to correlation between the light transmission through cell layers and the colony thickness, it is possible to measure the colony volume. As opposed to currently used techniques that measure colony area or diameter, differences in colony shape are thus taken into account, as shown on Supplementary Fig. 1. Comparison between growth rate measured by colony area and colony volume is shown on Supplementary Fig. 2, demonstrating a strong correlation (r = 0.94) between the two measures.

a) b)



Supplementary Fig. 1 Reconstruction of yeast colonies on an agar plate. Each pixel's intensity carries the information of the colony height at that position ("voxel"): The colony area is obtained directly from the 2D image, whereas the colony height is derived from the shades of grey that differ across the colony due to different thickness of the cell layer (and hence different light transmission). 3D reconstructions of the scanned plates with colonies were obtained using ImageJ software and its Interactive 3D surface plot plug-in (available from the ImageJ website: http://rsbweb.nih.gov/ij/). As shown on the figures (a – side view; b – top view), the resolution of the 2D scan is high enough to enable us a realistic view of the surface features of the colonies



Supplementary Fig. 2 Comparison between growth rate measured by colony area and colony volume. Colony area values were obtained using GenePix software and colony volume values were calculated as described in the text. Both types of values are shown as arbitrary units (AU). The results are shown separately for the biological duplicates of the SDL screen

Scanner detection noise and reproducibility; intensity quantification limit; reciprocal influence on colony growth

To determine the detection noise and quantification limit, several empty agar plates were scanned and processed as described (see below). The determined maximum noise is $1{,}25x10^6$ AU. We thus set the detection limit at $8.75x10^6$ AU. All colonies with intensities below $8.75x10^6$ AU were assigned an arbitrary value of $2x10^6$ AU and colour marked as "in the noise" in all subsequent analyses.

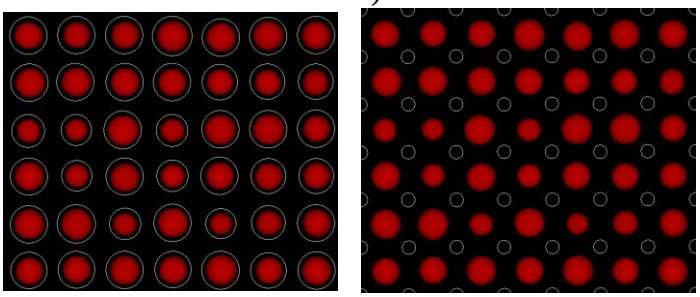
To determine the intensity detection reproducibility, the same agar plate was scanned several times and the resulting images analyzed. The average relative standard deviation was 0.3%, thus showing good reproducibility of the plate scanning and image analysis procedures.

To determine the reciprocal influence of colonies on growth, we replicated a plate with 384 colonies of the WT strain that contained several different patterns of missing colonies (modules) and determined the relative growth fitness (see below) of the colonies. The number of missing neighbours ranged from 1 up to 20. Missing neighbouring colonies significantly affected growth only in exceptional cases (modules with more than 8 missing neighbours) that did not occur in practice, thus we excluded this effect from growth fitness determination.

GenePix Pro image analysis

For each plate of the yeast knockout collection, a colony definition GAL file (GenePix Array List file) that describes block (plate) geometry, feature (colony) position and ID/Name (systematic/standard ORF name), was constructed. A separate background GAL file (common for all yeast knockout collection plates) with the same geometry was designed for the determination of background values. Any colonies whose growth was compromised due to agar imperfections (e.g. punctured agar, insoluble debris, contaminations) were flagged as absent and automatically marked accordingly in all subsequent analysis steps (see below). Compromised colonies of the wild-type strain were flagged as bad and excluded from subsequent analysis steps. All GAL files assume round shaped features which in the case of colony definition GAL files are fitted on plate images such that each feature completely surrounds the colony and includes also an empty perimeter around each colony (in this way no pixels belonging to the colony are excluded from the total intensity calculation). The background GAL file is fitted onto plate images so that none of the colony pixels fall within this background features (see Supplementary Fig. 3 a-b).

a) b)



Supplementary Fig. 3 After analysis of the plate images with correctly fitted both the colony definition and background GAL files we obtain two data files. The first data file generated with the colony definition GAL file contains the total feature intensities and number of pixels per feature. The second data file (generated with the background GAL file) contains the median pixel intensity for each background feature

Colony volume and relative growth fitness calculation

To automate and standardize the growth fitness calculation and minimize analysis time, we recorded an Excel spreadsheet based macro. The .txt (tab delimited) files obtained from GenePix Pro can be directly imported into Excel for subsequent analysis with the recorded macro. The analysis proceeds in 4 steps:

i) Background intensity calculation

For each feature of the background GAL file GenePix returns the median pixel intensity. For each colony we use four background features surrounding the colony to calculate the average median pixel intensity of the background. In this way we minimize the error due to local irregularities of the background. The average median pixel intensity is then multiplied with the number of pixels of the colony feature that are obtained with GenePix from the colony definition GAL file. This provides the total background intensity.

BgI_x = AVERAGE($Fm1_x$-$Fm4_x$) x PN_x,

where BgI_x is total background intensity of colony x, $Fm1_x$-$Fm4_x$ are the median pixel intensities for background features 1-4 of colony X, and PN_x is the number of pixels of colony X.

ii) Colony intensity calculation

The total intensity of the colony feature is composed of the intensity of the colony itself and the background intensity. After subtracting the calculated total background intensity from the total intensity of the colony feature the colony intensity is determined. This is roughly equal to the volume of the colony (see above)

$CI_x = TotFI_x - BgI_x \approx$ colony volume,

where CI_x is the intensity of colony X (≈ colony volume), $TotFI_x$ is the total intensity of the colony X feature, and BgI_x is total background intensity of colony X.

iii) Relative colony volume calculation

Each plate of the yeast knockout collection library contains 76 colonies of the wild-type strain. The median volume (colony intensity) of the wild-type strains is calculated for each plate separately. To get the relative colony volume of mutant strains, the colony intensity of each colony is then divided by the median WT volume. This normalization procedure allows the comparison of different plates and experiments.

$VrC_x = CI_x$ / MEDIAN($CI_{WT1-WT76}$),

where VrC_x is the relative volume of colony X, CI_x is the intensity of colony X (≈ colony volume), and $CI_{WT1-WT76}$ are the colony intensities of the 76 WT colonies on each yeast knockout collection plate.

iv) Relative growth fitness calculation
For each colony the relative growth fitness is obtained by dividing the relative colony volume of the colony grown on the test plate with the relative colony volume of the colony grown on the control plate
$R = VrC_x(test) / VrC_x (ctrl)$,
where R is the relative growth fitness, $VrC_x(test)$ is the relative colony volume of colony X grown on test plates, and $VrC_x(ctrl)$ is the relative colony volume of colony X grown on control plates.

Since SDL experiments are conducted in 786 format (two biological replicates on each plate), each replicate is analysed separately using the above described approach. This gives us two data sets with the relative growth fitness of all double mutants in the generated collection.

Double mutant growth fitness calculation
The above described protocol for relative growth fitness calculation is applicable both for chemical genomics and double mutant genetic interaction determination (SGA – synthetic genetic array analysis, SDL – synthetic dosage lethality assay).
In our SDL case, $VrC_x(test)$ and $VrC_x(ctrl)$ mean relative colony volume on double mutant selection plates containing galactose (PLA_2 expression) and relative colony volume on double mutant selection plates containing glucose (no PLA_2 expression), respectively.
To take into consideration the effect of galactose on growth of single mutants, we performed a galactose chemo-genomic experiment. The yeast knockout collection was sequentially pinned first on galactose containing plates and then on glucose containing control plates. The relative growth fitness of each single mutant was determined as described above. The calculated fitness was then used as a correction factor to obtain the final relative growth fitness of our double mutants.
At the end we obtained two data sets (for two biological replicates: i.e. two separate screens and in each screen two colonies represent every deletion strain) with the relative growth fitness of all ~4500 double mutants. All genes that were known to be involved in galactose metabolism, synthesis of histidine, arginine and lysine were excluded from the final data set. To score for the most significant genetic interactions, in the screen with automated scoring the threshold was set such that a final relative growth fitness lower than 0.4 (2.5x altered growth) in one biological repetition and a final relative growth fitness lower than 0.67 (1.5x altered growth) in the second biological repetition (or vice versa) was required, while in the visually inspected screen an obvious growth defect of both biological replicates was required.

Supplementary tables

Table S1 Growth fitness values for other members of the Rim101 pathway whose loss was found to increase sensitivity to induced PLA_2 expression in only one of our two screenings. In the first two results columns are given the growth fitness values from both SDL screens, either determined by visual inspection (1) or calculated as described in Supplementary methods (2) (relative growth fitness; R) for each gene. In the third results column, relative growth rates compared to the empty plasmid control and measured by growth curve determination are given. S – synthetic sick phenotype, L – synthetic lethal phenotype, VS – very synthetic sick phenotype, GN – grows normally (not significantly different from the wild-type control), NA – not determined due to slow growth on control medium[a] or technical difficulties[b]. * In the case of *RIM20,* only one colony out of two survived the SGA haploid selection procedure and therefore the result was not included in the main results set. Results interpreted as informative of negative genetic interaction are marked in bold

Gene	Summary of protein function	SDL screen		Growth curve
		1	2	
RIM20	Protein involved in proteolytic activation of Rim101p in response to alkaline pH; PalA/AIP1/Alix family member; interaction with the ESCRT-III subunit Snf7p suggests a relationship between pH response and multivesicular body formation	**VS**	**0.06***	**0.82**
RIM8	Protein of unknown function, involved in the proteolytic activation of Rim101p in response to alkaline pH; has similarity to A. nidulans PalF; essential for anaerobic growth	GN	**0.53**	**0.82**
RIM9	Protein of unknown function, involved in the proteolytic activation of Rim101p in response to alkaline pH; has similarity to A. nidulans PalI; putative membrane protein	NA[a]	**0.64**	0.99
RIM21	Component of the RIM101 pathway, has a role in cell wall construction and alkaline pH response; has similarity to A. nidulans PalH	**S**	NA[a]	**0.77**
VPS23	Component of the ESCRT-I complex, which is involved in ubiquitin-dependent sorting of proteins into the endosome; homologous to the mouse and human Tsg101 tumor susceptibility gene; mutants exhibit a Class E Vps phenotype	**L**	NA[a]	**0.87**
VPS25	Component of the ESCRT-II complex, which is involved in ubiquitin-dependent sorting of proteins into the endosome	**L**	NA[a]	0.94
VPS28	Component of the ESCRT-I complex (Stp22p, Srn2p, Vps28p, and Mvb12p), which is involved in ubiquitin-dependent sorting of proteins into the endosome; conserved C-terminal domain interacts with ESCRT-III subunit Vps20p	**L**	NA[a]	1.01
VPS37	Component of the ESCRT-I complex, which is involved in ubiquitin-dependent sorting of proteins into the endosome; suppressor of rna1-1 mutation; may be involved in RNA export from nucleus	GN	**0.48**	**0.80**
YGR122w	Probable ortholog of A. nidulans PalC, which is involved in pH regulation and binds to the ESCRT-III complex; null mutant does not properly process Rim101p and has decreased resistance to rapamycin; GFP-fusion protein is cytoplasmic	NA[b]	**0.73**	1.12