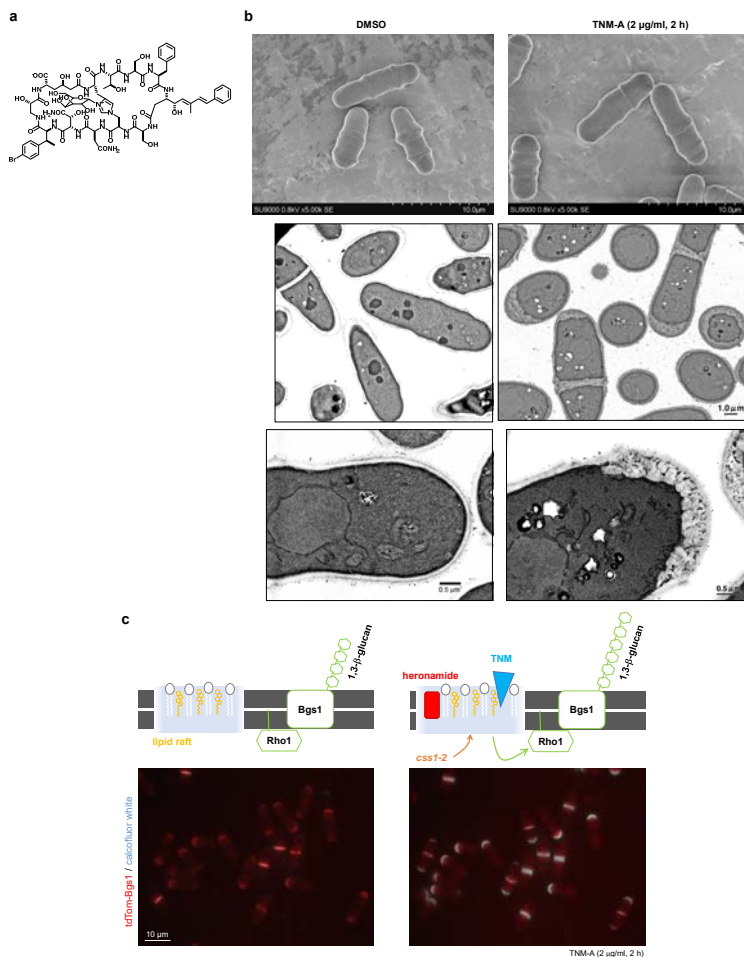


Summary

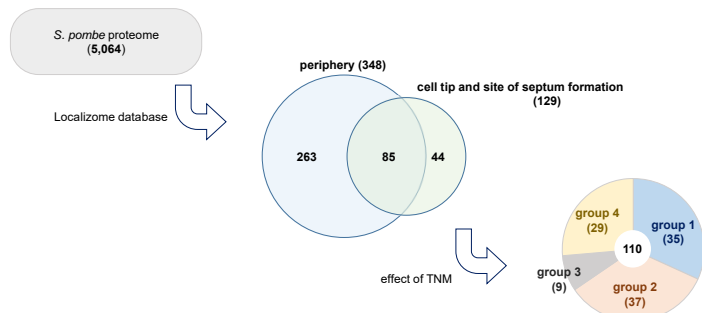
Fungal cell wall is one of the drug targets, yet the detailed molecular mechanism remains to be clarified. Theonellamides (TNMs) are marine sponge-derived peptides, targeting membrane sterols. They bind to cholesterol in mammalian cells, and ergosterol in yeast cells [1,2]. In fission yeast, TNMs induce accumulation of cell wall materials, in a Rho1- and Bgs1-dependent manner [1]. This phenomenon is unique to TNMs, and is not induced by traditional polyene antifungals. For understanding the molecular mechanisms of cell wall biosynthesis, we are investigating the molecular mechanism of the antifungal action of TNMs. To identify proteins whose subcellular localization is affected by TNM, and hopefully involved in the mode of action of TNM, we utilized the Localizome database which was established based on the cloned fission yeast ORFeome [3,4]. We focused on ~400 proteins, which were annotated to be localized at 'periphery' or 'cell tip and site of septum formation' in the Localizome database. Cellular localization of more than 100 proteins was changed by TNM-A; ~30 proteins were recruited to the sites with thick cell wall, while ~30 proteins disappeared from the plasma membrane to form cytoplasmic dots. In this presentation, we will show the detail of the screening results and would like to discuss the functional relationships between ergosterol and cell wall biosynthesis.

[1] Nishimura, S. et al. *Nat. Chem. Biol.* 2010, 6, 519. [2] Arita, Y. et al. *Chem. Biol.* 2015, 22, 604. [3] Matsuyama, A. et al. *Nat. Biotechnol.* 2006, 24, 841. [4] Shirai, A. et al. *J. Biol. Chem.* 2008, 283, 10745.

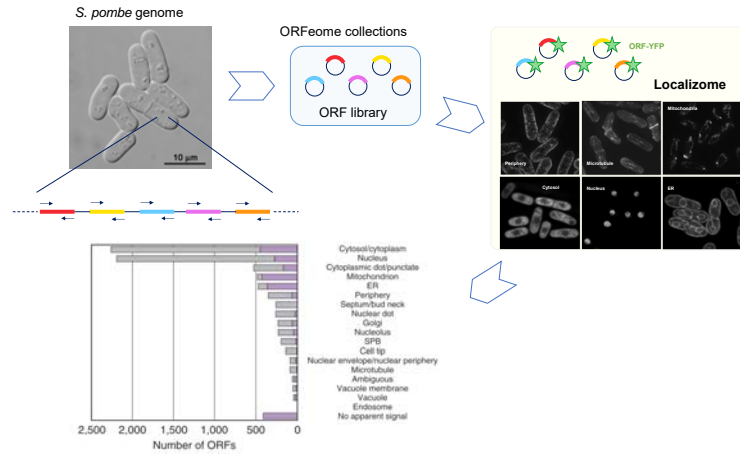
I. Sterol binding antifungal, theonellamide A (TNM-A).



II. Summary of the localizome screening.



ORFeome and localizome

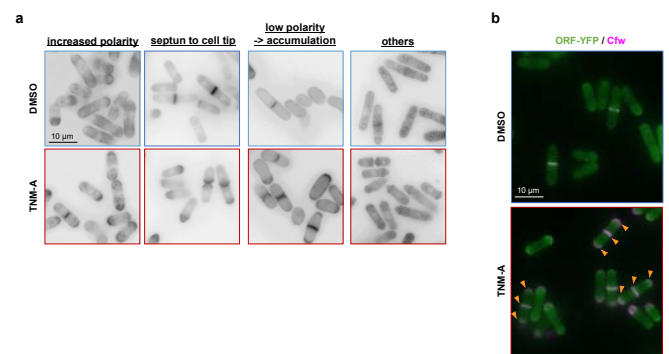


III. Proteins incorporated or not correctly delivered.

Gene ID	Gene name	Molecular Weight	Gene Product	No. of TM Domain
SPAC15A10.09c	pun1	31.4	SUR7 family protein Pun1 (predicted)	4
SPBC651.03c	gyp10	42.5	GTPase activating protein Gyp10	1
SPCC757.11c		51.9	transmembrane transporter (predicted)	12
SPBC1773.15	dai52	56	dipeptide transmembrane transporter Dai52 (predicted)	10
SPAC1002.16c		55.2	carboxylic acid transmembrane transporter (predicted)	10
SPCC320.08		56.7	transmembrane transporter (predicted)	10
SPCC417.10	dai51	56.6	dipeptide transmembrane transporter Dai51 (predicted)	12
SPBC15C4.04c		59.7	amino acid transmembrane transporter (predicted)	11
SPBC87.17		68.5	nucleobase transmembrane transporter (predicted)	10
SPAC183.15c		69	transmembrane transporter (predicted)	11
SPAC1F8.03c	str3	70.5	plasma membrane siderophore-iron transmembrane transporter Str3	13
SPBC484.13c	liz1	57.9	plasma membrane pantothenate transmembrane transporter Liz1	10
SPAC183.16c	vht1	62.8	plasma membrane vitamin H transmembrane transporter Vht1	12
SPAC922.08c	cat1	63.6	plasma membrane arginine/lysine amino acid transmembrane transporter Cat1	11
SPBC1271.10c		65.2	transmembrane transporter (predicted)	11
SPBC16A3.17c		65.2	transmembrane transporter (predicted)	13
SPBC649.04	lvi15	9.3	tail anchored plasma membrane protein Lvi15	-
SPAC4G9.07	mug133	60.3	UPF0300 family protein 2	-
SPBC359.03c	aat1	63.2	plasma membrane amino acid transmembrane transporter Aat1	11
SPAC1389.01c		64.5	nucleobase transmembrane transporter (predicted)	12
SPBC13A2.04c	ptr2	65.4	plasma membrane PTR family peptide transmembrane transporter Ptr2	12
SPAPB1A11.01	mfc1	55.3	prospore membrane copper transmembrane transporter Mfc1	10
SPAP7G5.06	per1	64	plasma membrane amino acid transmembrane transporter Per1	11

IV. Proteins accumulated at cell tips and/or septa.

Gene ID	Gene name	Molecular Weight	Gene Product	No. of TM Domain
SPAC17G8.10c	dma1	30.5	mitotic spindle checkpoint ubiquitin ligase Dma1	
SPBC83.18c	fic1	30.8	C2 domain protein Fic1	
SPAC5D6.09c	mug86	33.9	plasma membrane acetate transmembrane transporter (predicted)	6
SPCC1739.10	mug33	37	Tea1-interacting protein involved in exocytosis	4
SPCC1753.02c	gib3	53.8	G-protein coupled receptor Gib3	7
SPCP1E11.03	mug170	48.3	arrestin family Schizosaccharomyces specific protein Mug170	
SPBC23G7.08c	rga7	76.6	RhoGAP, GTPase activating protein Rga7	
SPBP887.27	mug30	93.4	HECT-type ubiquitin-protein ligase E3, found in association with TRAMP complex (predicted)	
SPCC1020.01c	pma2	110.1	F-type protein ATPase, P3-type Pma2	9
SPAC607.10	spo3	119.4	sporulation protein Spo3	
SPBC27.04	uds1	118.2	septation protein Uds1	
SPAC631.01c	accp2	29.8	F-actin capping protein beta subunit Acp2	
SPBC16E9.11c	pub3	89.2	HECT-type ubiquitin-protein ligase E3 Pub3 (predicted)	
SPAC11E3.02c	ync13	142.9	Munc family exocyst/endocyst regulator Ync13	
SPAC6F12.12	par2	72.4	protein phosphatase PP2A regulatory subunit B-56 Par2	
SPBC32H8.13c	mok12	266.5	prospore membrane alpha-1,3-glucan synthase Mok12	14



Notes.

This research was conducted with Mr. Akifumi Suginaga, Dr. Akihiko Matsuyama, and Prof. Minoru Yoshida. Cell wall analysis is being carried out in collaboration with Dr. Masako Ohsumi, Dr. Yoshihisa Namase, Ms. Vanessa S. D. Carvalho, Dr. Juan C. G. Cortés, and Dr. Juan. C. Ribas. The natural product is supplied by Prof. Shigeki Matsunaga.