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MINIREVIEWS

Modeling the Function of Bacterial Virulence Factors in *Saccharomyces cerevisiae*

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Our understanding of the molecular mechanisms of bacterial pathogenesis has been shaped largely by what we have learned from genetic screens. Transposon mutagenesis, in particular, has streamlined genetic analysis and led to the identification of a multitude of virulence factors in a wide range of bacterial pathogens (42). These mutant screens helped establish many of the paradigms in bacterial pathogenesis: two-component regulatory systems, pilus- and fimbria-mediated adhesion, type I to IV secretion systems, and pathogenicity islands (24).

However, the bacterium is only one variable in the complex equation that defines a host-pathogen interaction. Tractable genetic systems to study host responses to bacterial infections are largely lacking, presenting a stumbling block to the rapid identification and characterization of the eukaryotic targets of bacterial virulence factors. In overcoming these shortcomings, several research groups have embraced alternative host systems to model the interplay between a bacterium and its host (79). For example, the genetically tractable model organisms *Caenorhabditis elegans* and *Drosophila melanogaster* appear to mimic various aspects of mammalian disease and are susceptible to infection by a range of important pathogens (3, 21). Alternative strategies have used single-cell systems such as the *Dictyostelium* and *Acanthamoeba* spp. to examine the interaction of bacterial pathogens with individual cells (59, 76).

An increasingly popular model used to examine the function of individual virulence factors is the budding yeast *Saccharomyces cerevisiae*. Many of the molecular mechanisms regulating cellular processes that are affected during bacterial infection are relatively conserved from yeast to mammals, including DNA metabolism, programmed cell death (PCD), cell cycle control, cytoskeletal dynamics, and membrane traffic (8, 10, 23, 35, 37, 38, 81) (see Table 1 for examples of conserved cellular processes of particular relevance to bacterial pathogenesis). Thus, the appeal of yeast as a model system is clear: superb genetics, cell-free systems that reconstitute various cellular functions, a plethora of postgenomic tools, and a fully integrated genetic and phenotypic database spanning three decades of basic research (16). In this review, I summarize some of the recent examples of how several research groups are developing this powerful genetic system to identify and characterize the function of bacterial virulence factors. In addition, I briefly discuss some of the potential problems and challenges in using *S. cerevisiae* as a system to study microbial pathogenesis and when this model system can be of benefit to investigators.

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MODELING BACTERIUM-HOST INTERACTIONS IN YEAST

It is difficult to mimic in yeast the interactions between a whole pathogen and its host because of the relative infrequency of bacterial attachment to and invasion of a fungal cell. Although one report in the literature documents such an event, this interaction seems to be limited to the hyphal form (see below) (48). It is possible that the yeast cell wall represents a barrier too formidable for bacterial invasion or for the efficient delivery of toxins. Similarly, a productive pathogen-yeast interaction may not occur because the appropriate receptor(s) for bacterial adhesins and/or invasins is not present in yeast or because such receptor(s) are rendered inaccessible by the cell wall.

In theory, removal of the cell wall by enzymatic digestion could provide access to the underlying plasma membrane and permit adherence and/or invasion by a variety of eukaryotic pathogens. Indeed, for some DNA viruses such as bovine papillomavirus type I, removal of the cell wall is sufficient to permit attachment and entry into yeast cells (86, 87). Unfortunately, in order to maintain structural integrity, yeast spheroplasts must be maintained in an osmotic stabilizer (e.g., 1 M sorbitol). It is not clear how these conditions may affect the expression or delivery bacterial virulence factors. Therefore, the inability of bacterial pathogens to attach or invade yeast spheroplasts may not necessarily reflect a lack of receptors or the machinery required for attachment or invasion but instead be an artifact of the experimental conditions.

One fungal system has been described to model a host–bacterial-pathogen interaction. Hogan and Kolter have shown that *Pseudomonas aeruginosa* efficiently attaches to and kills the pathogenic fungus *Candida albicans* (48). Interestingly, this interaction is restricted to the hyphal but not the yeast form of *C. albicans*. It is not clear why the hyphal stage of growth is more sensitive to bacterial killing but it is possible that hypha-specific cell surface factors permit more efficient bacterial attachment. The fungicidal activity of *Pseudomonas* was depen-

TABLE 1. Conservation among eukaryotic cellular processes relevant in bacterial pathogens[a]

Cellular process	Molecular components in:		Associated pathogen(s)	Review(s) (reference)
	Mammals	Yeast		
Cytoskeleton				
Actin dynamics	Rho proteins	Rho proteins	*Salmonella*, *Yersinia*, *Pseudomonas*, EPEC, and *Listeria* spp.	23, 26, 36, 51
	RhoGAP and GEF	Cdc24p, Rom1-3p, Bem		
	Arp2/3 complex	Arp2/3 complex		
	Type 1 myosins	Myo3p, Myo5p		
	Fimbrin	Sac6p		
	AAK, BIK	Ark1p, Prk1		
	WASP	Las17p		
Microtubule dynamics	CDC42	Rho/Cdc42p	*Campylobacter*, *Chlamydia*, and *Shigella* spp.	34, 37, 85
	Dynein	Dyn1-3p		
	Dynactin	Nip100p		
	MDia	Bni1p		
	APC (?)	Kar9p		
	EB1	Bim1p		
Membrane traffic				
ER transport	Coatamer	COPI	*Legionella* and *Brucella spp*	28, 52, 72
	COPII	COPII		
	hSar1	Sar1p		
	ARF1	Arf1p		
	ARF GAP and GEF	ARF GAP and GEF		
	Rabs	Rab1p		
Endosomal transport	Hrs, TSG101	ESCRT I to III	*Salmonella*, *Mycobacteria*, *Legionella*, and *Chlamydia* spp.	51, 54, 58, 64
	PIKFYVE	Fab1p		
	HVps34	Vps34p		
	Clathrin	Clathrin		
	AP-1 and AP-3	AP-1 and AP-3		
	Rabs	Ypt6p, Ypt31-32p		
	Syntaxins	Pep12p, Tlg1p, Tlg2p		
Endocytosis	Clathrin	Clathrin	*Salmonella*, *Shigella*, and *Listeria* spp.	19, 23
	AP-2, AP180	AP-2, AP180A		
	Hip1	Sal2p		
	Epsin	Ent1-2p		
	Synaptojanin	Inp51-53p		
	Amphiphysisn	Rsv		
Autophagy	Tor pathway	Tor kinases	*Legionella* and *Salmonella* spp.	22, 33, 45
	Beclin 1	Apg6p		
	p150	Apg14		
	U1k1	Apg1p		
	MAP1LC3	Apg8p		

[a] The information provided highlights the conservation between yeast and mammalian cells in cellular pathways relevant to bacterial pathogenesis. The examples provided are covered in greater detail in the indicated reviews. EPEC, enteropathogenic *E. coli*.

dent on a subset of the virulence factors important for infection of mammalian cells, particularly those involved in biofilm formation (48). However, it is not clear whether other virulence factors (e.g., proteins secreted by *P. aeruginosa* type III secretion system) can modulate interactions with *C. albicans*. It is possible that some *Pseudomonas* factors may have evolved to inhibit the growth of eukaryotic competitors in their natural habitats and have subsequently become part of the

TABLE 2. Expression of bacterial virulence factors in *S. cerevisiae*

Bacterial protein	Activity in:		Reference(s)
	Mammalian or plant cells	Yeast	
YopE	RhoGAP	RhoGAP	55, 83
ExoT	RhoGAP and ADP ribosylation	RhoGAP and cytotoxicity	27
SptP	RhoGAP and tyrosine phosphatase	RhoGAP and ND[a]	70
SopE2	RhoGEF	RhoGEF	70
SipA	Binding of F-actin	Binding of F-actin	55
YpkA	Plasma membrane S/T kinase	Plasma membrane	52, 55
YopM	Nuclear localization	Nuclear localization	11, 55, 78
ExoU	Lipase	Lipase	68, 73
CdtB	DNA damage and/or cell cycle arrest	DNA damage and/or cell cycle arrest	41
YopJ	Inhibition of MAP kinase signaling	Inhibition of MAP kinase signaling	84
AvrPtoB	Inhibition of PCD	Inhibition of H_2O_2-mediated cell death	1
HopPtoE-G	Inhibition of PCD	Inhibition of Bax-induced cell death	50

[a] ND, not determined.

repertoire of virulence factors that can be used by these organisms during opportunistic infections.

MODELING THE FUNCTION OF BACTERIAL VIRULENCE FACTORS IN YEAST

Although it is not currently feasible to model in yeast the macro-interactions between bacterial pathogens and mammalian cells, it is possible to characterize the function of individual virulence factors or toxins by expressing them in yeast (Table 2).

Inhibitors of cytoskeletal function. Many of the virulence factors exported by invasive or adherent bacterial pathogens target regulators of cytoskeletal assembly (36). Because the basic principles controlling cytoskeletal dynamics are conserved among eukaryotic cells (6, 15), yeast provides an attractive system in which to model the function of these bacterial toxins. In pioneering studies, Lesser and Miller (55) and Wolf-Watz and coworkers (83) characterized the function of the *Yersinia enterocolitica* cytotoxin YopE in yeast cells. YopE is one of several proteins injected by *Yersinia* directly into the cytoplasm of infected mammalian cells and is required for the localized disruption of the actin network (4). YopE is homologous to a family of bacterial virulence factors that act as GTPase activating proteins (GAP) on the mammalian Rho family of small G proteins (4, 15).

Underscoring the functional conservation of cytoskeletal function, the expression of YopE in yeast was highly toxic and, as predicted from studies in mammalian cells, cytotoxicity could be attributed to defects in actin dynamics (55, 83). In yeast, the actin cytoskeleton is mostly present as actin cables and cortical patches (6). In small budded cells, actin cables are aligned toward sites of new growth and cortical patches are polarized toward the new bud (6). YopE expression did not depolymerize these actin structures but caused phenotypes indicative of a loss of control in actin reorganization (55). Cortical patches failed to polarize to sites of active growth, actin rings failed to form at the bud-neck region and displayed defective cytokinesis (55). A likely reason for why yeast cells are highly susceptible to YopE expression is because interference with the actin cytoskeleton triggers a morphogenesis checkpoint, leading to an arrest in nuclear division (56).

The interference of YopE with actin dynamics in yeast requires GAP activity since mutants lacking a crucial arginine residue at the putative active site are no longer cytotoxic (55). In yeast there are six members of the Rho family of GTPases: Rho1 to Rho5 and Cdc42p (5). One of the best-characterized Rho-regulated pathways in yeast controls cell wall integrity. Upon cell wall perturbation, the GTP exchange factors (GEF) Rom1p and Rom2p activate Rho1p by exchanging GDP with GTP. Rho1p-GTP, in turn, activates the atypical protein kinase C (Pkc1p) (43). Pkc1p phosphorylates and activates Bck1p, the first kinase in a mitogen-activated protein (MAP) kinase cascade required for the transcription of genes involved in maintaining cell wall integrity (39, 43). Wolf-Watz and coworkers showed that the cytopathic effects of YopE expression are suppressed by overexpression of activators of Rho1p (the GEF Rom2p) or downstream effectors of Rho1p-dependent signaling (Bck1p) (83). In addition, overexpression of Ste20p, a kinase that can activate Bck1p independently of Rho1p, also partially rescued YopE cytotoxicity (83). These results suggest that inhibition of a Rho1p-regulated pathway is responsible for YopE-mediated toxicity. Interestingly, overexpression of mammalian RhoA, a close homologue of Rho1p, and activated forms of mammalian Rac1 and Cdc42 also suppressed the cytotoxic effects of YopE (83), suggesting that the function of these Rho proteins is conserved between yeast and mammals. It should be pointed out that although Rho1p is one of the targets of YopE in yeast and is responsible for its cytotoxic effect, it is unlikely to be the only target. In mammalian cells, YopE shows GAP activity on multiple GTPases, including Cdc42, RhoA, and Rac (12).

Small G proteins appear to be common targets of bacterial virulence factors (2). *Salmonella enterica* injects two proteins into mammalian cells, SopE2 and SptP, which act as a GEF and a GAP, respectively, for Rho proteins (88). During infection, SopE2 has been postulated to initiate the actin rearrangements required for bacterial invasion, whereas SptP reverses the SopE2-mediated stimulation of Rho proteins after entry (26). In yeast, SopE2 expression activated a series of Cdc42p-dependent MAP kinases, most notably the filamentous growth MAP kinase Kss1p (70). A less-pronounced activation of MAP kinases of the pheromone response and the cell wall integrity pathway was also observed (70). It is not clear whether the simultaneous activation of these pathways reflects cross talk among upstream regulators of the various MAP kinase pathways or SopE2's activation of multiple Rho proteins. The availability of well-characterized conditional alleles of *cdc42* in yeast (31, 60) should allow for a rigorous characterization of the molecular function of SopE2.

In contrast, SptP may specifically down regulate Cdc42p in yeast (70). Molina and coworkers have shown that the hyperactivation of Kss1p in yeast strain lacking the Cdc42p GAPs Bem3 and Rga2p was suppressed by expression of SptP (70). In addition to GAP-dependent modulation of Rho protein function, other virulence factors possess other enzymatic activities that can inhibit actin dynamics. *P. aeruginosa* secretes two closely related toxins, ExoS and ExoT, which possess both Rho GAP and ADP-ribosyltransferase activities (2, 9). Engel and coworkers have recently shown that expression of either the GAP or the ADP-ribosylation domains inhibited growth in yeast, indicating that both domains independently contribute to cytotoxicity (27). Indeed, expression of either domain in mammalian cells led to actin cytoskeleton disruption (27). These results suggest that a target of the ADP-ribosylation activity of ExoT is a regulator of actin dynamics, and this target is probably conserved in yeast.

Other virulence factors directly target the actin cytoskeleton. For example *Salmonella* sp. SipA (SspA) modulates actin dynamics in mammalian cells that (46). In vitro, SspA binds filamentous actin and inhibits its depolymerization (57). In yeast, SipA colocalized with cortical patches and actin cables (55). These actin structures became resistant to the depolymerizing effects of latrunculin A, a monomeric actin-binding drug, suggesting that SipA stabilizes yeast actin structures in vivo (55).

The analysis of cytoskeleton-disrupting bacterial factors in yeast is particularly well suited for genetic and proteomic analysis. Large collections of conditional alleles of various regulators and components of the actin cytoskeleton and compre-

hensive maps of their genetic and protein-protein interactions (32) should provide attractive systems for identifying targets of these virulence factors.

Inhibitors of DNA metabolism. A novel theme in cellular microbiology is the ability of pathogens to interfere with the cell cycle of host cells (44). Several unrelated pathogens (e.g., *Campylobacter* sp., *Escherichia coli* isolates, *Shigella* sp., and *Haemophilus ducreyi*) secrete a cytolethal distending toxin (CDT) that blocks cell cycle progression in mammalian cells at G_2/M (20, 65). CDT is composed of three subunits: CdtA, CdtB, and CdtC. CdtB alone appears to be responsible for toxicity since its expression in mammalian cells recapitulates the effects of CDT (53). CdtB shares limited homology with nucleases but shows very weak DNase activity in vitro (53).

Pickett and coworkers demonstrated that CdtB has genotoxic activity in yeast (41). By combining flow cytometric analysis of DNA content and morphological analysis of cells expressing CtdB, Pickett's group concluded that yeast cells, like mammalian cells, arrested at the G_2/M transition point in the cell cycle (41). It was possible that CtdB caused cell cycle arrest either by inducing DNA damage or by activating signaling pathways that sense DNA damage. To distinguish between these possibilities, Pickett's group tested whether *mec1* mutants (encoding an ATM-like protein responsible for inducing the G_2/M checkpoint) (71) arrested at G_2/M during CtdB intoxication. Because *mec1* mutants were still sensitive to CtdB, it is likely that the observed toxicity is due to direct DNA damage rather than activation of proteins involved in sensing damage (41). In support of these observations, prolonged expression of CtdB in yeast cells led to fragmented nuclei and chromosomal degradation. Furthermore, expression of *RNE2*, a DNA damage-inducible gene, was upregulated during CtdB expression and CtdB toxicity was abolished by mutations in the putative nuclease active site (41). Therefore, the CDTs appear to induce cell cycle arrest by directly damaging DNA. However, it is not clear why CtdB-induced DNA damage does not activate a G_1/S checkpoint arrest or what is the Mec1-independent mechanism that senses this damage. A yeast model system for CtdB could significantly streamline the molecular analysis of this problem by taking advantage of the large number of conditional mutants available for studying DNA damage in yeast (13).

Inhibitors of membrane structure and function. In addition to manipulating the cytoskeleton, various intracellular and extracellular pathogens have managed to develop mechanisms to manipulate the endomembrane system of mammalian cells (51, 58). The consequences of these manipulations can range from the selective arrest of vesicular and endosomal traffic to the inactivation of signaling lipids to the degradation of membranes.

P. aeruginosa secretes a range of proteins (ExoS, ExoT, ExoY, and ExoU) directly into the cytoplasm of mammalian cells. ExoU is particularly cytotoxic and *Pseudomonas* strains expressing this protein are associated with accelerated lung injury and increased development of septic shock (74). Given the high toxicity of this protein, it has been difficult to perform functional analysis in transiently transfected mammalian cells. To address these shortcomings, Rabin and Hauser (68) and Frank and coworkers (73) used yeast as a model system to gain a better understanding of ExoU function. Consistent with observations in mammalian cells, yeast cells rapidly lost viability after expression of ExoU. Frank's group isolated spontaneous suppressors of ExoU toxicity, but the mutations were restricted to alterations or rearrangements within ExoU itself (73). These results suggest that ExoU may act on multiple targets making the isolation of single suppressor mutations rare. To further understand how ExoU functions, the morphology of ExoU-expressing yeast cells was carefully examined, resulting in the demonstration that ExoU fragmented vacuolar membranes (73). Fragmentation of vacuolar membranes can occur as a result of a block in protein and membrane transport to vacuoles or by disruption of vacuolar components (18). To identify the affected pathways, Frank's group tested a range of pharmacological inhibitors of lipases, proteases, and vacuolar function in ExoU-expressing yeast cells. Interestingly, a subclass of lipase inhibitors (haloenol lactone suicide substrate and methyl arachidonyl fluorophosphates) could inhibit ExoU-mediated toxicity in yeast (73). These inhibitors also relieved ExoU-dependent cytotoxicity during *P. aeruginosa* infection of mammalian cells (73).

This lipase activity is consistent with the presence of a weak but conserved patatin-like phospholipase domain in ExoU (47). This family has a conserved GXSXG serine hydrolase motif and DX-G/A active site (47). Disruption of the catalytic dyad abolished ExoU toxicity in yeast and in mammalian cells (73). The lipolytic activity of ExoU in yeast has broad substrate specificity; both neutral lipids and phospholipids are degraded. Interestingly, recombinant ExoU did not display lipase activity on synthetic liposomes unless it was coincubated with yeast extracts (73). This suggests that ExoU requires eukaryotic factor(s) to achieve full biochemical activity. Therefore, even though the initial screen for ExoU-resistant yeast failed to yield extragenic suppressors (73), mutations in these factor(s) should be forthcoming and will help elucidate the molecular mechanism of ExoU function.

Inhibitors of MAP kinase signaling and PCD. Members of the YopJ/Avr family of proteins show structural similarities to adenoviral cysteine proteases and ubiquitin-like protein proteases (63). YopJ/Avr proteins inhibit intracellular signaling required for inflammatory cytokine secretion (mammals) or for the activation of PCD (mammals and plants). For example, in *Yersinia* sp., YopJ inhibits the phosphorylation and activation of MAP kinase kinases and the IκB kinase β (62).

Orth and coworkers have recently shown that expression of YopJ in yeast cells led to a decreased response to mating pheromones, lowered mating efficiencies, and increased sensitivity to high salt concentrations and sorbitol (84). These phenotypes are characteristic of impaired MAP kinase signaling cascades required either in the mating response or for growth under hypo-osmotic conditions (39). Indeed, YopJ expression reduced the levels of phosphorylated Fus3p and Hog1p (targets of these MAP kinase pathways during exposure to pheromones and sorbitol, respectively) and suppressed the lethality of *snl1* mutants, an osmolarity sensor that inhibits Hog1p activation (84). These results suggest that virulence proteins such as YopJ target common structural components of eukaryotic MAP kinases cascades even though the outcomes of these pathways may not be necessarily conserved.

In plant immunity, resistance to bacterial pathogens begins with the recognition of a bacterial protein (avirulence protein

[Avr]) by a plant protein (resistance protein [R]) (17). In response to an Avr-R protein interaction, plants cells activate the hypersensitive response (HR). HR is characterized by rapid cell death that bears all of the hallmarks of PCD and whose ultimate goal is to limit the spread of the pathogen (17). Some plant pathogens bypass the HR response by producing factors that inhibit PCD. For example, *Pseudomonas syringae* produces two proteins, AvrPto and AvrPtoB, that are recognized by the R protein Pto. The AvrPto-Pto interaction elicits an HR response. In contrast, the AvrPtoB-Pto interaction does not cause an HR response but also inhibits the HR response elicited by AvrPto-Pto (1). AvrPtoB is conserved among very diverse plant pathogens, suggesting that AvrPtoB is a ubiquitous mechanism enhancing infectivity by inhibiting PCD and the HR response.

Like most eukaryotic cells, yeast undergoes PCD in response to oxidative stress or expression of mammalian proapoptotic factors such as Bax (14). PCD in yeast, as in mammalian apoptosis, includes cytochrome *c* release, DNA fragmentation, and chromatin condensation (14). However, there is considerable debate as to whether yeast undergo true apoptotic events as opposed to an autophagy-dependent death (33, 66). Martin and coworkers have shown that AvrPtoB suppresses PCD in yeast cells in response to proapoptotic conditions such as oxidative stress or extreme heat shock (1). In contrast to plants, AvrPtoB did not suppress Bax-induced cell death in yeast cells (1). Although these results may reflect important differences between plant and fungal PCD, one must be wary of overexpression artifacts. For example, if Bax and AvrPtoB are not expressed at similar levels, true phenotypic suppression may be missed. A similar observation has been made for multicopy suppressors of YopE function. Expression of YopE from the weak *MET3* promoter, but not the stronger *GAL1* promoter, was efficiently suppressed by overexpression of Rho activators (55, 83).

More recently, Alfano and coworkers have shown that a new set of Avr proteins—AvrPrphEpTo, HopPtoG, HoptoF, and HopPtoE—suppress PCD in plant and yeast cells (50). Interestingly, these Avr proteins can suppress Bax-mediated but not peroxide-induced PCD in yeast (50). These results highlight the diversity in potential targets of various Avr proteins and their relative conservation among eukaryotic cells.

Subcellular tropism in yeast. Other bacterial virulence factors have been expressed in yeast, but their function has not been fully characterized. For example, Lesser and Miller have reported that the *Yersinia* Ser/Thr kinase YpkA localizes to the plasma membrane after expression in yeast cells (55). YpkA translocated by *Yersinia* during infection also localizes to the plasma membrane (40). Another *Yersinia* virulence factor, YopM, a member of the Leu-rich-repeat family of proteins (LRR), localizes to the nucleus of mammalian cells during infections (77). YopM expressed in yeast also localizes to the nucleus, indicating that other bacterial factors are not required for YopM nuclear import (55, 78). Two research groups have independently used yeast to determine the molecular requirements for YopM transport into the nucleus (11, 78). Through deletion analysis, Straley's group demonstrated that the amino-terminal LRR region of YopM was required for YopM translocation into the yeast nucleus (78). The Cornelis group showed that the last 32 amino acids of the YopM were sufficient for nuclear localization of a heterologous protein in both yeast and mammalian cells. Interestingly, this acidic peptide is similar to the nuclear localization signal from the influenza virus nucleoprotein, suggesting a conserved mechanism of nuclear import (11).

Surprisingly, in *sec18* temperature-sensitive mutants, which are conditionally defective in membrane fusion, YopM accumulated in the cytoplasm (78). Since Sec18p is required at various stages of membrane traffic, Straley's group concluded that YopM transport to the yeast nucleus requires vesicular transport. Although this is possible, it should be pointed out that prolonged blocks in protein secretion (>2 h) in yeast lead to a phenomena termed an arrest in secretion response (ASR) (61). The consequences of ASR are varied and include a block in transcription and translation, inhibition in nuclear import, and redistribution of nuclear proteins (61). Therefore, additional study is required to elucidate whether YopM indeed defines a novel vesicle-mediated transport pathway to the eukaryotic nucleus. Nonetheless, because the import of YopM into the yeast nucleus appears to be independent of the major yeast nuclear importins (11), it would not be surprising if YopM utilizes a novel mechanism for accumulation in the eukaryotic nucleus.

YEAST FUNCTIONAL GENOMICS AND VIRULENCE GENE IDENTIFICATION

In the last few years, postgenomic tools in yeast have advanced at a breathtaking pace (16). The generation of comprehensive protein-protein interaction maps (75), maps detailing all known genetic interactions (7), global surveys of subcellular localization of all yeast proteins (49), and the availability of strain collections where all nonessential open reading frame (ORF) have been epitope tagged or deleted (67) have revolutionized how researchers approach biological questions in yeast and how experiments are designed and executed (7). For a thorough discussion of these tools, the reader is directed to a number of recent reviews (16, 25, 30, 67, 80, 82).

Functional genomic approaches have made yeast an organism of choice to model basic disease-related processes involving cell cycle control, signal transduction, cytoskeletal dynamics, and protein transport (81). Therefore, with the basic premise that some bacterial virulence factors can elicit similar responses in yeast and mammalian cells, it is clear that investigators will soon take full advantage of the genetic, biochemical, and genomic tools available in yeast to identify the targets of bacterial toxins. Lau and coworkers, for example, have recently used the yeast gene deletion collection to identify mutants that were either more susceptible or resistant to the *P. aeruginosa* phenazine pyocyanin (PCN) (69). Lau's group found that mutations that compromised the function of the yeast vacuolar proton ATPase (either directly or by affecting protein sorting and vesicular transport) were particularly sensitive to PCN (69). The genome-wide screen in yeast led Lau's group to test whether v-ATPases are the target of PCN in mammalian cells. Interestingly, the mammalian v-ATPases, like their yeast counterparts, appear to be inhibited by reactive oxygen intermediates produced in response to PCN (69). These experiments illustrate how yeast functional genomics can be readily adapted to facilitate the identification of mam-

malian processes affected by a bacterial toxin. With the advent of robotic systems and mass transformation protocols, we predict that genome-scale suppression and/or epistatic analysis in yeast can be readily adapted to create short lists of potential targets of a wide range of bacterial virulence factors. Some potential applications of yeast genetic and genomic tools to identify bacterial virulence factors and their corresponding targets in eukaryotic cells include the following targeted screens for virulence factors: (i) toxicity (the major cellular process is disrupted, e.g., membrane traffic), (ii) conditional phenotypes (the sensitivity or resistance to various environmental conditions, drugs, etc., can highlight the disruption of particular cellular pathways, e.g., some cytoskeletal functions), (iii) reporter gene fusions (assessment of the signaling pathways, e.g., MAP kinase signaling), and (iv) reporter protein fusions (the activity of reporter protein can be used to monitor the disruption of protein localization machinery, e.g., organelle targeting). Genome-wide approaches to identify disrupted cellular pathways include the following: (i) yeast collection of TAP (tandem-affinity purification)-, glutathione *S*-transferase- or FLAG-tagged ORFs (this can be used to screen for yeast proteins that bind to bacterial proteins) (30), (ii) yeast collection of green fluorescent protein-tagged ORFs (29) (to determine mislocalization of key organelle markers), (iii) synthetic lethal screens, i.e., the expression of bacterial protein in strain collections of haploid gene deletions (~4,000 ORFs; nonessential) or a heterozygous diploid strain collection (~6,000 ORFs) (a mutation in some cellular pathway may sensitize the strain to growth defects induced by the bacterial toxin; this type of analysis can also be performed in a yeast strain collection where each ORF deletion is bar coded with a unique probe; loss of fitness can be monitored by the loss of probe signal in DNA microarrays [30]), (iv) phenomic analysis of recombinant yeast strains (comparison of the growth kinetics of recombinant yeast strains to a set of known yeast strains), and (v) transcriptional profiling (genes induced or repressed in response to bacterial protein expression can be used to identify affected cellular pathways).

Yeast expression systems can also be used to identify novel virulence factors in less-well-understood pathogens. Our laboratory is currently using yeast as a tool to identify virulence factors from the obligate intracellular pathogen *Chlamydia trachomatis* (unpublished data). Because chlamydiae are not currently amenable to genetic manipulation, our understanding of the molecular basis of how these pathogens coopt the eukaryotic host is limited. By screening yeast libraries expressing all *Chlamydia* ORFs of unknown function, we can identify and cluster putative bacterial virulence factors by the phenotypes they induce in yeast cells. Quantitative phenotyping can also be used to characterize the eukaryotic pathways affected in these recombinant strains by identifying mutants from ordered yeast deletion sets that match the phenotypic fingerprint induced by expression of bacterial toxin.

IS YEAST THE SYSTEM FOR ME?

Before launching into using yeast as a model system to study a toxin or virulence factor of interest, one should critically assess whether the expected target molecule(s) are conserved among eukaryotes. Although the internal architecture of yeast and mammals share many molecular features, an additional layer of complexity is needed to build and maintain a multicellular organism. For example, the proteins and factors required to coordinate intercellular communication, control tissue formation and specialization, regulate cell death, and build innate and adaptive immunity are not present in fungal cells. If one's pathogen of interest targets any of these specialized processes, it is unlikely that yeast will provide a good model system. Furthermore, it is also possible that even when some cellular functions are conserved (e.g., SNAREs in membrane fusion), individual molecular components are different enough to be recognized by bacterial toxins (e.g., clostridia neurotoxins).

Another potential concern in modeling the function of individual bacterial virulence factors is that these proteins are being expressed at nonphysiological levels and in the absence of other factors normally present during infection. Virulence proteins that are posttranslationally processed in the bacterium, inserted into a membrane by a bacterium-encoded apparatus (e.g., type III secretion system), or part of a multiprotein complex may not retain biological activity when expressed in yeast. The expression of integral membrane proteins can be particularly problematic. Membrane proteins without the appropriate targeting signals are prone to aggregation or subjected to posttranslational modifications and/or degradation. Therefore, one should exercise caution when assigning a role to a bacterial factor based on phenotypes induced in yeast.

Since the first description of using yeast to characterize bacterial factors, relatively few reports have fully explored the use of this system. Most of these studies confirmed, and in few cases extended, what was already known about the molecular mechanism of a few select model virulence factors. Very few research articles have moved past the "proof-of-principle" concept and made full use of yeast genetic, biochemical, and genomic tools to either identify of fully dissect the function of a novel virulence factor. Most bacterial pathogenesis researchers may be wary of investing the energy and time required to navigate through the vast yeast cell biology and genetics literature. Therefore, although the possibility of performing genetics and genome-scale studies in a host system is appealing, it may not be sufficiently enticing, especially when one is not certain as to whether the findings will be relevant to mammalian pathogenesis. A potential short-term solution is to establish collaborations with the yeast research community, especially those interested in cytoskeletal and membrane dynamics, a common target of bacterial virulence factors. In addition, many research institutions are building resource centers for genome sciences, which often include tools for functional genomics in model organisms. In the near future, researchers should have access to core facilities that will aid with the design and implementation of genome-wide screens in yeast with the same ease as for other yeast-based technologies (e.g., two-hybrid screens). Nonetheless, the interpretation of results obtained from such experiments will still require a basic knowledge of yeast cell biology. Fortunately, the yeast research community is rapidly moving toward a systems biology approach where much of our current understanding of yeast cell biology is available as cross-referenced, searchable public databases (e.g., www.yeastgenome.org, yeast.cellzome.com, and mips.gsf.de).

The full potential of yeast as a tool for virulence gene discovery and eukaryotic target identification remains to be realized. Modeling the interaction of bacterial virulence factors with eukaryotic cellular pathways in yeast should further allow the implementation of simple cell-based assays to screen for inhibitors of these interactions (82). These methodologies can be adapted to design new generations of antimicrobial agents that prevent the association of virulence factors with their respective eukaryotic targets. In the process we can gain new insights into both the basic mechanisms of microbial pathogenesis and the eukaryotic cell biology.

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REFERENCES

1. **Abramovitch, R. B., Y. J. Kim, S. Chen, M. B. Dickman, and G. B. Martin.** 2003. *Pseudomonas* type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. EMBO J. **22:**60–69.
2. **Aktories, K., G. Schmidt, and I. Just.** 2000. Rho GTPases as targets of bacterial protein toxins. Biol. Chem. **381:**421–426.
3. **Alegado, R. A., M. C. Campbell, W. C. Chen, S. S. Slutz, and M. W. Tan.** 2003. Characterization of mediators of microbial virulence and innate immunity using the *Caenorhabditis elegans* host-pathogen model. Cell Microbiol. **5:**435–444.
4. **Andor, A., K. Trulzsch, M. Essler, A. Roggenkamp, A. Wiedemann, J. Heesemann, and M. Aepfelbacher.** 2001. YopE of *Yersinia*, a GAP for Rho GTPases, selectively modulates Rac-dependent actin structures in endothelial cells. Cell Microbiol. **3:**301–310.
5. **Arellano, M., P. M. Coll, and P. Perez.** 1999. RHO GTPases in the control of cell morphology, cell polarity, and actin localization in fission yeast. Microsc. Res. Tech. **47:**51–60.
6. **Ayscough, K. R., and D. G. Drubin.** 1996. ACTIN: general principles from studies in yeast. Annu. Rev. Cell Dev. Biol. **12:**129–160.
7. **Bader, G. D., A. Heilbut, B. Andrews, M. Tyers, T. Hughes, and C. Boone.** 2003. Functional genomics and proteomics: charting a multidimensional map of the yeast cell. Trends Cell Biol. **13:**344–356.
8. **Ballatori, N., and A. R. Villalobos.** 2002. Defining the molecular and cellular basis of toxicity using comparative models. Toxicol. Appl. Pharmacol. **183:** 207–220.
9. **Barbieri, J. T.** 2000. *Pseudomonas aeruginosa* exoenzyme S, a bifunctional type-III secreted cytotoxin. Int. J. Med. Microbiol. **290:**381–387.
10. **Barr, M. M.** 2003. Super models. Physiol. Genomics **13:**15–24.
11. **Benabdillah, R., L. J. Mota, S. Lutzelschwab, E. Demoinet, and G. R. Cornelis.** 2004. Identification of a nuclear targeting signal in YopM from *Yersinia* spp. Microb. Pathog. **36:**247–261.
12. **Black, D. S., and J. B. Bliska.** 2000. The RhoGAP activity of the *Yersinia pseudotuberculosis* cytotoxin YopE is required for antiphagocytic function and virulence. Mol. Microbiol. **37:**515–527.
13. **Broomfield, S., T. Hryciw, and W. Xiao.** 2001. DNA postreplication repair and mutagenesis in *Saccharomyces cerevisiae*. Mutat. Res. **486:**167–184.
14. **Burhans, W. C., M. Weinberger, M. A. Marchetti, L. Ramachandran, G. D'Urso, and J. A. Huberman.** 2003. Apoptosis-like yeast cell death in response to DNA damage and replication defects. Mutat. Res. **532:**227–243.
15. **Burridge, K., and K. Wennerberg.** 2004. Rho and Rac take center stage. Cell **116:**167–179.
16. **Castrillo, J. I., and S. G. Oliver.** 2004. Yeast as a touchstone in post-genomic research: strategies for integrative analysis in functional genomics. J. Biochem. Mol. Biol. **37:**93–106.
17. **Collmer, A.** 1998. Determinants of pathogenicity and avirulence in plant pathogenic bacteria. Curr. Opin. Plant Biol. **1:**329–335.
18. **Conibear, E., and T. H. Stevens.** 1998. Multiple sorting pathways between the late Golgi and the vacuole in yeast. Biochim. Biophys. Acta **1404:**211–230.
19. **Cossart, P., and P. J. Sansonetti.** 2004. Bacterial invasion: the paradigms of enteroinvasive pathogens. Science **304:**242–248.
20. **De Rycke, J., and E. Oswald.** 2001. Cytolethal distending toxin (CDT): a bacterial weapon to control host cell proliferation? FEMS Microbiol. Lett. **203:**141–148.
21. **Dionne, M. S., and D. S. Schneider.** 2002. Screening the fruitfly immune system. Genome Biol. **3:**REVIEWS1010. [Online.] http://genomebiology.com/2002/3/4/reviews/1010.
22. **Dorn, B. R., W. A. Dunn, Jr., and A. Progulske-Fox.** 2002. Bacterial interactions with the autophagic pathway. Cell Microbiol. **4:**1–10.
23. **Engqvist-Goldstein, A. E., and D. G. Drubin.** 2003. Actin assembly and endocytosis: from yeast to mammals. Annu. Rev. Cell Dev. Biol. **19:**287–332.
24. **Finlay, B. B., and S. Falkow.** 1997. Common themes in microbial pathogenicity revisited. Microbiol. Mol. Biol. Rev. **61:**136–169.
25. **Friesen, H., J. S. Millman, and B. J. Andrews.** 2003. Yeast functional genomics and cell biology: no longer in the dark. Genome Biol. **4:**352. [Online.] http://genomebiology.com/2003/4/12/352.
26. **Galan, J. E., and D. Zhou.** 2000. Striking a balance: modulation of the actin cytoskeleton by *Salmonella*. Proc. Natl. Acad. Sci. USA **97:**8754–8761.
27. **Garrity-Ryan, L., S. Shafikhani, P. Balachandran, L. Nguyen, J. Oza, T. Jakobsen, J. Sargent, X. Fang, S. Cordwell, M. A. Matthay, and J. N. Engel.** 2004. The ADP-ribosyltransferase domain of *Pseudomonas aeruginosa* ExoT contributes to its biological activities. Infect. Immun. **72:**546–558.
28. **Gaynor, E. C., T. R. Graham, and S. D. Emr.** 1998. COPI in ER/Golgi and intra-Golgi transport: do yeast COPI mutants point the way? Biochim. Biophys. Acta **1404:**33–51.
29. **Ghaemmaghami, S., W. K. Huh, K. Bower, R. W. Howson, A. Belle, N. Dephoure, E. K. O'Shea, and J. S. Weissman.** 2003. Global analysis of protein expression in yeast. Nature **425:**737–741.
30. **Giaever, G., A. M. Chu, L. Ni, C. Connelly, L. Riles, S. Veronneau, S. Dow, A. Lucau-Danila, K. Anderson, B. Andre, A. P. Arkin, A. Astromoff, M. El-Bakkoury, R. Bangham, R. Benito, S. Brachat, S. Campanaro, M. Curtiss, K. Davis, A. Deutschbauer, K. D. Entian, P. Flaherty, F. Foury, D. J. Garfinkel, M. Gerstein, D. Gotte, U. Guldener, J. H. Hegemann, S. Hempel, Z. Herman, D. F. Jaramillo, D. E. Kelly, S. L. Kelly, P. Kotter, D. LaBonte, D. C. Lamb, N. Lan, H. Liang, H. Liao, L. Liu, C. Luo, M. Lussier, R. Mao, P. Menard, S. L. Ooi, J. L. Revuelta, C. J. Roberts, M. Rose, P. Ross-Macdonald, B. Scherens, G. Schimmack, B. Shafer, D. D. Shoemaker, S. Sookhai-Mahadeo, R. K. Storms, J. N. Strathern, G. Valle, M. Voet, G. Volckaert, C. Y. Wang, T. R. Ward, J. Wilhelmy, E. A. Winzeler, Y. Yang, G. Yen, E. Youngman, K. Yu, H. Bussey, J. D. Boeke, M. Snyder, P. Philippsen, R. W. Davis, and M. Johnston.** 2002. Functional profiling of the *Saccharomyces cerevisiae* genome. Nature **418:**387–391.
31. **Gladfelter, A. S., J. J. Moskow, T. R. Zyla, and D. J. Lew.** 2001. Isolation and characterization of effector-loop mutants of CDC42 in yeast. Mol. Biol. Cell **12:**1239–1255.
32. **Goode, B. L., and A. A. Rodal.** 2001. Modular complexes that regulate actin assembly in budding yeast. Curr. Opin. Microbiol. **4:**703–712.
33. **Gozuacik, D., and A. Kimchi.** 2004. Autophagy as a cell death and tumor suppressor mechanism. Oncogene **23:**2891–2906.
34. **Grieshaber, S. S., N. A. Grieshaber, and T. Hackstadt.** 2003. *Chlamydia trachomatis* uses host cell dynein to traffic to the microtubule-organizing center in a p50 dynamitin-independent process. J. Cell Sci. **116:**3793–3802.
35. **Gruenberg, J., and H. Stenmark.** 2004. The biogenesis of multivesicular endosomes. Nat. Rev. Mol. Cell. Biol. **5:**317–323.
36. **Gruenheid, S., and B. B. Finlay.** 2003. Microbial pathogenesis and cytoskeletal function. Nature **422:**775–781.
37. **Gundersen, G. G.** 2002. Evolutionary conservation of microtubule-capture mechanisms. Nat. Rev. Mol. Cell. Biol. **3:**296–304.
38. **Gundersen, G. G., and A. Bretscher.** 2003. Cell biology. Microtubule asymmetry. Science **300:**2040–2041.
39. **Gustin, M. C., J. Albertyn, M. Alexander, and K. Davenport.** 1998. MAP kinase pathways in the yeast *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **62:**1264–1300.
40. **Hakansson, S., E. E. Galyov, R. Rosqvist, and H. Wolf-Watz.** 1996. The *Yersinia* YpkA Ser/Thr kinase is translocated and subsequently targeted to the inner surface of the HeLa cell plasma membrane. Mol. Microbiol. **20:** 593–603.
41. **Hassane, D. C., R. B. Lee, M. D. Mendenhall, and C. L. Pickett.** 2001. Cytolethal distending toxin demonstrates genotoxic activity in a yeast model. Infect. Immun. **69:**5752–5759.
42. **Hayes, F.** 2003. Transposon-based strategies for microbial functional genomics and proteomics. Annu. Rev. Genet. **37:**3–29.
43. **Heinisch, J. J., A. Lorberg, H. P. Schmitz, and J. J. Jacoby.** 1999. The protein kinase C-mediated MAP kinase pathway involved in the maintenance of cellular integrity in *Saccharomyces cerevisiae*. Mol. Microbiol. **32:**671–680.
44. **Henderson, B., M. Wilson, and J. Hyams.** 1998. Cellular microbiology: cycling into the millennium. Trends Cell Biol. **8:**384–387.
45. **Hernandez, L. D., M. Pypaert, R. A. Flavell, and J. E. Galan.** 2003. A *Salmonella* protein causes macrophage cell death by inducing autophagy. J. Cell Biol. **163:**1123–1131.
46. **Higashide, W., S. Dai, V. P. Hombs, and D. Zhou.** 2002. Involvement of SipA in modulating actin dynamics during *Salmonella* invasion into cultured epithelial cells. Cell Microbiol. **4:**357–365.
47. **Hirschberg, H. J., J. W. Simons, N. Dekker, and M. R. Egmond.** 2001. Cloning, expression, purification and characterization of patatin, a novel phospholipase A. Eur. J. Biochem. **268:**5037–5044.
48. **Hogan, D. A., and R. Kolter.** 2002. *Pseudomonas-Candida* interactions: an ecological role for virulence factors. Science **296:**2229–2232.
49. **Huh, W. K., J. V. Falvo, L. C. Gerke, A. S. Carroll, R. W. Howson, J. S. Weissman, and E. K. O'Shea.** 2003. Global analysis of protein localization in budding yeast. Nature **425:**686–691.

50. **Jamir, Y., M. Guo, H. S. Oh, T. Petnicki-Ocwieja, S. Chen, X. Tang, M. B. Dickman, A. Collmer, and R. A. J.** 2004. Identification of *Pseudomonas syringae* type III effectors that can suppress programmed cell death in plants and yeast. Plant J. **37:**554–565.
51. **Knodler, L. A., J. Celli, and B. B. Finlay.** 2001. Pathogenic trickery: deception of host cell processes. Nat. Rev. Mol. Cell. Biol. **2:**578–588.
52. **Kreis, T. E., M. Lowe, and R. Pepperkok.** 1995. COPs regulating membrane traffic. Annu. Rev. Cell Dev. Biol. **11:**677–706.
53. **Lara-Tejero, M., and J. E. Galan.** 2000. A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein. Science **290:**354–357.
54. **Lemmon, S. K., and L. M. Traub.** 2000. Sorting in the endosomal system in yeast and animal cells. Curr. Opin. Cell Biol. **12:**457–466.
55. **Lesser, C. F., and S. I. Miller.** 2001. Expression of microbial virulence proteins in *Saccharomyces cerevisiae* models mammalian infection. EMBO J. **20:**1840–1849.
56. **Lew, D. J.** 2000. Cell-cycle checkpoints that ensure coordination between nuclear and cytoplasmic events in *Saccharomyces cerevisiae*. Curr. Opin. Genet. Dev. **10:**47–53.
57. **McGhie, E. J., R. D. Hayward, and V. Koronakis.** 2004. Control of actin turnover by a salmonella invasion protein. Mol. Cell **13:**497–510.
58. **Meresse, S., O. Steele-Mortimer, E. Moreno, M. Desjardins, B. Finlay, and J. P. Gorvel.** 1999. Controlling the maturation of pathogen-containing vacuoles: a matter of life and death. Nat. Cell Biol. **1:**E183–E188.
59. **Moffat, J. F., and L. S. Tompkins.** 1992. A quantitative model of intracellular growth of *Legionella pneumophila* in *Acanthamoeba castellanii*. Infect. Immun. **60:**296–301.
60. **Mosch, H. U., T. Kohler, and G. H. Braus.** 2001. Different domains of the essential GTPase Cdc42p required for growth and development of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **21:**235–248.
61. **Nanduri, J., and A. M. Tartakoff.** 2001. The arrest of secretion response in yeast: signaling from the secretory path to the nucleus via Wsc proteins and Pkc1p. Mol. Cell **8:**281–289.
62. **Orth, K., L. E. Palmer, Z. Q. Bao, S. Stewart, A. E. Rudolph, J. B. Bliska, and J. E. Dixon.** 1999. Inhibition of the mitogen-activated protein kinase kinase superfamily by a *Yersinia* effector. Science **285:**1920–1923.
63. **Orth, K., Z. Xu, M. B. Mudgett, Z. Q. Bao, L. E. Palmer, J. B. Bliska, W. F. Mangel, B. Staskawicz, and J. E. Dixon.** 2000. Disruption of signaling by *Yersinia* effector YopJ, a ubiquitin-like protein protease. Science **290:**1594–1597.
64. **Pelham, H. R.** 2002. Insights from yeast endosomes. Curr. Opin. Cell Biol. **14:**454–462.
65. **Pickett, C. L., and C. A. Whitehouse.** 1999. The cytolethal distending toxin family. Trends Microbiol. **7:**292–297.
66. **Priault, M., N. Camougrand, K. W. Kinnally, F. M. Vallette, and S. Manon.** 2003. Yeast as a tool to study Bax/mitochondrial interactions in cell death. FEMS Yeast Res. **4:**15–27.
67. **Que, Q. Q., and E. A. Winzeler.** 2002. Large-scale mutagenesis and functional genomics in yeast. Funct. Integr. Genomics **2:**193–198.
68. **Rabin, S. D., and A. R. Hauser.** 2003. *Pseudomonas aeruginosa* ExoU, a toxin transported by the type III secretion system, kills *Saccharomyces cerevisiae*. Infect. Immun. **71:**4144–4150.
69. **Ran, H., D. J. Hassett, and G. W. Lau.** 2003. Human targets of *Pseudomonas aeruginosa* pyocyanin. Proc. Natl. Acad. Sci. USA **100:**14315–14320.
70. **Rodriguez-Pachon, J. M., H. Martin, G. North, R. Rotger, C. Nombela, and M. Molina.** 2002. A novel connection between the yeast Cdc42 GTPase and the Slt2-mediated cell integrity pathway identified through the effect of secreted *Salmonella* GTPase modulators. J. Biol. Chem. **277:**27094–27102.
71. **Rouse, J., and S. P. Jackson.** 2002. Interfaces between the detection, signaling, and repair of DNA damage. Science **297:**547–551.
72. **Roy, C. R.** 2002. Exploitation of the endoplasmic reticulum by bacterial pathogens. Trends Microbiol. **10:**418–424.
73. **Sato, H., D. W. Frank, C. J. Hillard, J. B. Feix, R. R. Pankhaniya, K. Moriyama, V. Finck-Barbancon, A. Buchaklian, M. Lei, R. M. Long, J. Wiener-Kronish, and T. Sawa.** 2003. The mechanism of action of the *Pseudomonas aeruginosa*-encoded type III cytotoxin, ExoU. EMBO J. **22:**2959–2969.
74. **Schulert, G. S., H. Feltman, S. D. Rabin, C. G. Martin, S. E. Battle, J. Rello, and A. R. Hauser.** 2003. Secretion of the toxin ExoU is a marker for highly virulent *Pseudomonas aeruginosa* isolates obtained from patients with hospital-acquired pneumonia. J. Infect. Dis. **188:**1695–1706**.**
75. **Schwikowski, B., P. Uetz, and S. Fields.** 2000. A network of protein-protein interactions in yeast. Nat. Biotechnol. **18:**1257–1261.
76. **Skriwan, C., M. Fajardo, S. Hagele, M. Horn, M. Wagner, R. Michel, G. Krohne, M. Schleicher, J. Hacker, and M. Steinert.** 2002. Various bacterial pathogens and symbionts infect the amoeba *Dictyostelium discoideum*. Int. J. Med. Microbiol. **291:**615–624.
77. **Skrzypek, E., C. Cowan, and S. C. Straley.** 1998. Targeting of the *Yersinia pestis* YopM protein into HeLa cells and intracellular trafficking to the nucleus. Mol. Microbiol. **30:**1051–1065.
78. **Skrzypek, E., T. Myers-Morales, S. W. Whiteheart, and S. C. Straley.** 2003. Application of a *Saccharomyces cerevisiae* model to study requirements for trafficking of *Yersinia pestis* YopM in eucaryotic cells. Infect. Immun. **71:**937–947.
79. **Steinert, M., M. Leippe, and T. Roeder.** 2003. Surrogate hosts: protozoa and invertebrates as models for studying pathogen-host interactions. Int. J. Med. Microbiol. **293:**321–332.
80. **Steinmetz, L. M., and R. W. Davis.** 2004. Maximizing the potential of functional genomics. Nat. Rev. Genet. **5:**190–201.
81. **Steinmetz, L. M., C. Scharfe, A. M. Deutschbauer, D. Mokranjac, Z. S. Herman, T. Jones, A. M. Chu, G. Giaever, H. Prokisch, P. J. Oefner, and R. W. Davis.** 2002. Systematic screen for human disease genes in yeast. Nat. Genet. **31:**400–404.
82. **Tucker, C. L.** 2002. High-throughput cell-based assays in yeast. Drug Discov. Today. **7:**S125–S130.
83. **Von Pawel-Rammingen, U., M. V. Telepnev, G. Schmidt, K. Aktories, H. Wolf-Watz, and R. Rosqvist.** 2000. GAP activity of the *Yersinia* YopE cytotoxin specifically targets the Rho pathway: a mechanism for disruption of actin microfilament structure. Mol. Microbiol. **36:**737–748.
84. **Yoon, S., Z. Liu, Y. Eyobo, and K. Orth.** 2003. *Yersinia* effector YopJ inhibits yeast MAPK signaling pathways by an evolutionarily conserved mechanism. J. Biol. Chem. **278:**2131–2135.
85. **Yoshida, S., and C. Sasakawa.** 2003. Exploiting host microtubule dynamics: a new aspect of bacterial invasion. Trends Microbiol. **11:**139–143.
86. **Zhao, K. N., and I. H. Frazer.** 2002. Replication of bovine papillomavirus type 1 (BPV-1) DNA in *Saccharomyces cerevisiae* following infection with BPV-1 virions. J. Virol. **76:**3359–3364.
87. **Zhao, K. N., and I. H. Frazer.** 2002. *Saccharomyces cerevisiae* is permissive for replication of bovine papillomavirus type 1. J. Virol. **76:**12265–12273.
88. **Zhou, D., and J. Galan.** 2001. *Salmonella* entry into host cells: the work in concert of type III secreted effector proteins. Microbes Infect. **3:**1293–1298.