Functional genomics in the study of yeast cell polarity: moving in the right direction

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The budding yeast Saccharomyces cerevisiae has been used extensively for the study of cell polarity, owing to both its experimental tractability and the high conservation of cell polarity and other basic biological processes among eukaryotes. The budding yeast has also served as a pioneer model organism for virtually all genome-scale approaches, including functional genomics, which aims to define gene function and biological pathways systematically through the analysis of high-throughput experimental data. Here, we outline the contributions of functional genomics and high-throughput methodologies to the study of cell polarity in the budding yeast. We integrate data from published genetic screens that use a variety of functional genomics approaches to query different aspects of polarity. Our integrated dataset is enriched for polarity processes, as well as some processes that are not intrinsically linked to cell polarity, and may provide new areas for future study.

1. Introduction

Cell asymmetry, or polarity, refers to spatial differences in shape, structure or function of cellular components. Cell polarity is a defining feature of almost all cells, and is used differently based on cell type to modulate cell behaviour and define distinct cellular domains. The budding yeast Saccharomyces cerevisiae is an attractive model for studying the establishment of cell polarity for two main reasons: (i) core biological processes in S. cerevisiae are conserved in higher eukaryotic cells, allowing inference of function; and (ii) yeast is an experimentally tractable organism that is amenable to genetic manipulation [1].

The field of functional genomics aims to define gene (and protein) functions and interactions, using data derived from genome-scale experiments. As noted above, model organisms like yeast have been essential for annotating gene function and for developing tools and approaches that have driven major advances in functional genomics and genome biology. In this review, we highlight research that has made use of functional genomics approaches to study polarity in S. cerevisiae. We then describe general trends identified through the analysis of 35 genome-wide genetic screens from different groups, which suggest that there are several essential biological processes required for cell polarity in yeast. Finally, we discuss our view of future directions for cell polarity research using functional genomics approaches in yeast. It is beyond the scope of this review to give a detailed description of the biology of polarity in yeast, but we refer the reader to excellent reviews recently published by the YeastBook project [2,3].

Saccharomyces cerevisiae cells become polarized during three discrete phases: budding, mating (shmoo formation) and filamentous growth. Each of these modes of polarized cell growth is regulated by different spatio-temporal and biological cues, but all hinge on a common series of molecular polarity determinants beginning with the small guanosine triphosphatase (GTPase) Cdc42. Budding is internally induced at the time of cell cycle commitment in late G1 (figure 1a), which is regulated by the cyclin-dependent protein kinase Cdc28 [4–6]. Cortical spatial landmarks inherited from the previous round of cell division determine the bud site and lead to activation of small GTPase molecules and their regulators: Rsr1, Cdc24 and Cdc42 (figure 1b). Active cycling of Rsr1 GTPase between GDP- and GTP-bound forms is hypothesized to concentrate the
activated form of Cdc42 and Cdc24, the guanine nucleotide-exchange factor (GEF) of Cdc42, at the selected bud site [2,7,8]. Polarization of Cdc42 triggers the switch from isotropic, symmetrical growth to non-symmetrical growth along an axis dictated by the septins and the actin cytoskeleton [5,9]. First, Cdc42 activates Bni1, a formin that together with Bnr1 assembles the filamentous (F) actin cables [10]. Polarized F-actin cables function as tracks along which the myosin motor, Myo2, moves exocytic vesicles from the Golgi [11,12], as well as numerous other organelles [13–16], to the incipient bud site, promoting growth of the bud. The polarisome complex (consisting of Spa2, Pea2, Bni1, Bnr1) promotes this actin-mediated exocytosis by coupling the extension of F-actin cables with vesicle fusion [17]. Cortical actin patches are also polarized in response to Cdc42 activation and are nucleated by the highly conserved Arp2/3 complex following its activation by the Cdc42-regulated kinases, Cla4 and Ste20 [18,19]. Actin patches play an integral role in endocytosis, allowing for regulation of lipid and protein levels at the membrane of the emerging bud [20]. Second, septins are recruited to the bud neck following activation of the Rho-like GTPases Gic1 and Gic2 by Cdc42, and form a ring-shaped cytoskeletal complex that associates with the plasma membrane, providing a diffusion barrier between the mother and the bud [21,22]. The septins also provide a scaffold upon which the contractile actomyosin ring is assembled. During telophase, triggered by the mitotic exit network, the septin hourglass shape splits into two rings, surrounding the cytokinetic machinery [23]. As the actomyosin ring contracts, the cytoskeletal and exocytic machinery, which earlier in the cell cycle promoted bud growth, is redirected from the bud cortex to the bud neck, delivering post-Golgi vesicles to the actomyosin ring [24].

Alternatively, polarity in both mating and filamentous growth is induced by external cues. Mating is initiated upon exposure to mating pheromone from cells of the opposite mating type, which leads to the outgrowth of a mating projection, or ‘shmoo’. The same Cdc42-based machinery used in budding drives polarized cell growth and shmoo formation during mating. In addition to regulating polarized secretion, Cdc42 also regulates the mating MAPK signalling pathway to turn on mating-specific genes required for cell fusion and the cyclin-dependent kinase inhibitor Far1, leading to G1 arrest.

Figure 1. An overview of Cdc42 regulation during polarized bud growth. (a) Budding occurs when the cell switches from isotropic to apical cell growth, leading to the formation of a bud. (b) Bud-site selection protein Rsr1 activates the GEF Cdc24, which converts Cdc42 to its active GTP-bound state. Activated Cdc42 binds to numerous effectors, promoting actin patch nucleation, actin cable assembly and septin/actomyosin ring assembly.

Figure 2. An overview of Cdc42 regulation during mating. (a) Mating begins after exposure to mating pheromone from cells of the opposite mating type, which leads to the outgrowth of a mating projection, or ‘shmoo’. (b) The same Cdc42-based machinery used in budding drives polarized cell growth and shmoo formation during mating. In addition to regulating polarized secretion, Cdc42 also regulates the mating MAPK signalling pathway to turn on mating-specific genes required for cell fusion and the cyclin-dependent kinase inhibitor Far1, leading to G1 arrest.
be discovered. Below, we summarize functional genomics efforts that aim to uncover new regulators of cell asymmetry and polarized growth in *S. cerevisiae*.

2. High-throughput methods for polarity studies

The ease of genetic manipulation in *S. cerevisiae* has enabled the creation of a wealth of large-scale collections of strains with deleted [29,30], hypomorphic [31–34], tagged [35–37] or over-expressed genes [38–43], as well as the development of new methods for performing cost-effective and straightforward systematic analyses. Here, we give an overview of methodological advances in the fields of yeast genomics, microscopy and proteomics that have contributed to our understanding of cell polarity (figure 4).

(a) Genetic assays

Yeast researchers have used forward genetic screens productively for many years to discover regulators of cell polarity. For example, *CDC42* was first identified in classical genetic screens for temperature-sensitive mutants that arrest their cell cycle with a uniform morphological phenotype [19,44]. More recently, so-called reverse genetic approaches, which involve assessment of the phenotypic consequences of a known genetic mutation, have provided a means to immediately link genotype to phenotype. The budding yeast heterozygous deletion collection is composed of a set of diploid yeast strains in which each of the approximately 6000 genes is individually deleted and replaced with a drug resistance cassette [29,30]. The deletion collection was the first genome-scale reagent produced for reverse genetics screens and was used to generate the haploid non-essential deletion collection (consisting of strains harbouring deletion mutations in 80% of yeast genes), inspiring the development of numerous methods for the manipulation of these collections. In particular, synthetic genetic array (SGA) analysis automates yeast genetics and has enabled high-throughput genetic studies in yeast. The SGA method involves a set of replica pinning and serial selection steps, allowing facile introduction of any marked allele into any set of arrayed strains in a high-throughput manner [45].

A major application of SGA analysis has involved systematic assessment of genetic interactions (GIs) between two partial or complete loss-of-function alleles [46–53]. A GI can be defined as an unexpected deviation in double mutant growth rate, using colony size as a proxy for cellular fitness [54]. A negative GI, in which the double mutant has a fitness defect than would be predicted based on the fitness of the two single mutants, suggests that the two genes have a redundant role as components of parallel pathways. A positive GI, in which the double mutant is more severe fitness defect than would be predicted based on the fitness of the two single mutants, suggests that the two genes have a redundant role as components of parallel pathways. A positive GI, in which the double mutant is more severe fitness defect than would be predicted based on the fitness of the two single mutants, suggests that the two genes have a redundant role as components of parallel pathways. A positive GI, in which the double mutant is more fit than expected, suggests that the two gene products may function in the same pathway. A global survey of GIs between approximately 5.4 million gene pairs revealed an interesting relationship between GIs and the essentiality of protein complex members; genes encoding components of non-essential complexes show predominantly positive GIs, whereas negative GIs are more often found among genes encoding components of essential complexes [55]. This observation suggests that essential complexes contain internal redundancy, allowing retention of function after loss of a single complex member. Additionally, GI profiles (the set of

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**Figure 3.** An overview of Cdc42 regulation during filamentous growth. (a) Filamentous growth occurs when cells elongate and exhibit enhanced cell adhesion, leading to a switch from yeast cell form to filamentous form. (b) Filamentation occurs when nitrogen starvation is sensed by Sho1 and Msb2, leading to the activation of Cdc42 and the downstream activation of the MAPK pathway. This leads to both cell elongation via a G2 delay as well as the expression of the flocculin Flo11 and other adhesion proteins.
GIs for a particular gene) can be used to infer gene function through a ‘guilt by association’ principle of analysis: genes that have similar GIs are likely to encode proteins that are part of the same pathway or complex. The first proof-of-principle work validating SGA analysis as a method for mapping synthetic lethal (negative) GIs included a focus on cell polarity genes and revealed new components of pathways known to regulate actin cable nucleation [45]. Alternative methods of assaying GIs involve competitive growth assays of pooled yeast strains (for example, deletion mutants) that each contain a unique ‘molecular barcode’. Flanked by common PCR priming sequences, all barcodes can be amplified simultaneously, allowing for analysis by either microarray hybridization or sequencing [56–61].

Other types of GIs have proved useful for exploring cell polarity pathways. In particular, complex haploinsufficiency (CHI) occurs when a heterozygous double mutant exhibits a more severe phenotype than the corresponding heterozygous single mutants. CHI is thought to identify genes that function within the same pathway or structure and provides useful insight in predicting multigenic influences in human disorders. CHI has been heavily used to explore GIs specific to strains deleted for genes that encode components of the endosomal sorting complex required for transport (ESCRT), leading to an unusual relocalization of F-actin to prevacuolar endocytic compartments called E-bodies [62]. Although actin’s involvement with endocytosis is well established, this discovery marked the first instance of F-actin accumulation on E-bodies. Interestingly, CHI screens have also drawn a convincing link between the actin cytoskeleton and several proteasome components including numerous members of both the 19S regulatory particle and the 20S core particle. This observation suggests that loss of proteolytic activity may indirectly affect actin and cell polarity, and that there may in fact be a direct physical interaction between the proteasome and actin filaments [63].

Genetic interactions caused by changes in gene dosage have also been systematically explored using gene overexpression libraries. Synthetic dosage lethality (SDL), wherein overproduction of one gene product exacerbates a mutant phenotype, has been used to identify kinase targets whose regulated phosphorylation is important for cell polarity [52,64]. For example, follow-up of an SDL screen revealed that phosphorylation of Rga2, a GTPase-activating protein for Cdc42, by the cyclin-dependent protein kinase Pho85 inhibits Rga2 activity to ensure appropriate activation of Cdc42 during cell polarity establishment [64]. Dosage suppression, wherein overproduction of one gene rescues a mutant phenotype of another gene, often reveals downstream components of the pathway and has been used productively by many groups to study cell polarity pathways [65–68]. Finally, numerous polarity screens have assessed the effects of single gene perturbations, either mutation or overexpression, in specific conditions including chemical or environmental stress [69–75].

In addition to colony size, more specific colony-based assays have been used to discover genes involved in polarity and morphogenesis. For example, regulators of the mating pathway have been identified by screening yeast mutant strains for defects in activation of a relevant reporter gene [76–79]. Additionally, as filamentous growth is in essence a colony phenotype distinguished by filaments of interconnected cells, screens for colony morphology have been used with great success to implicate genes in filamentous differentiation [3,80–88]. Interestingly, the vast majority of mutants identified in colony morphology screens do not show compromised fitness [87], highlighting the importance of morphology-based assays as a complement to fitness-based assays to uncover cell polarity mechanisms.

(b) Microscopy-based assays
In order to fully dissect the spatio-temporal dynamics of the intricate biological systems controlling cell polarity, numerous qualitative and quantitative cellular imaging and analysis techniques have been developed, which can be
used alongside the sophisticated genetics outlined in §2a. As polarized growth is characterized by distinct subcellular changes in morphology, systematic image-based approaches to directly observe cell morphology during budding, shmoo formation and hyphal outgrowth are very powerful. Global analysis of protein localization in *S. cerevisiae* is possible owing to the availability of an enormously useful collection of strains each expressing a unique endogenously tagged green fluorescent protein (GFP)-fusion protein [35,36]. The original analysis of this collection identified almost 250 proteins that localize to the primary compartments involved in polarization (bud, bud neck, actin cytoskeleton and cell periphery), comprising approximately 4% of the total number of tagged proteins [35]. So far, the GFP library has seen limited use for exploring cell polarity pathways—one screen identified proteins that localize to the mating projection in pheromone-treated cells [89]. A more common approach has been to study changes in the localization of cell polarity-specific proteins fused to a fluorescent moiety in the deletion collection or in the context of gene overexpression [53,90–98]. For instance, the deletion mutant array was screened for mislocalization of modified Snc1-GFP, yeast synaptobrevin, to identify novel regulators of its internalization [53].

While extremely useful for live-imaging, fusion to a fluorescent protein tag may lead to mislocalization of some proteins owing to sterical hindrance or inhibition of critical protein–protein interactions, or even reorganization of subcellular complexes [35]. Also, multimerization of the fluorescent protein moiety can lead to artefactual formation of foci [99]. These effects can be minimized by carefully choosing the size and position of fluorescent labels, and selecting those that best complement corresponding loss-of-function alleles in phenotypic assays or in known GIs, for example synthetic lethality. One alternative to fluorescently tagging full-length proteins is to use fluorescent dyes that bind to specific proteins or subcellular compartments. For instance, rhodamine–phalloidin binds to F-actin and is commonly used to visualize the actin cytoskeleton. Calcofluor-white, which binds to chitin in the cell wall and highlights cell shape and bud scars, has been successfully used to identify genes involved in polarized budding [91]. Lastly, the lipophilic dye FM4-64 can be used to track endocytosis and endosomal trafficking in live cells [92]. Despite various technical challenges, microscopy-based assays remain the premier method for gaining insights into subcellular morphological changes.

**3. Analysis and synthesis of genetic screens**

As summarized above, many functional genomics approaches have been used to study cell polarity in yeast. We wondered whether particular approaches were more suited for the analysis of different aspects of cell polarity—for example, are specific types of genes best identified with a particular method (cell biological as compared to fitness-based readout, for example), and are different polarity processes best queried with a certain approach (budding as compared to filamentation)? To begin to address these questions, we aimed to compile an inclusive, unbiased list of existing genome-wide datasets that either assessed the GIs between genes involved in different aspects of cell polarity or analysed chemo-GIs using compounds with known effects on the processes that establish cell polarity. A survey of the literature uncovered 35 published works from numerous groups (see electronic supplementary material, table S1), which included data on 3492 unique open reading frames (roughly 58% of the entire genome). As a filtering metric only those genes that were observed in four or more screens were integrated into our polarity network (n = 485; figure 5 and electronic
supplementary material, table S2). We used GO Slim enrichment analysis [118] to identify biological processes that were over-represented in this dataset. As expected, the dataset was significantly enriched for genes with annotated roles in processes essential for cellular polarization, including cytoskeletal organization \((p < 5.9 \times 10^{-4})\), cell wall organization \((p < 2.9 \times 10^{-14})\), cellular membrane organization \((p < 9.4 \times 10^{-8})\) and transport \((p < 1.5 \times 10^{-8})\). The dataset was also enriched for genes with roles in a number of processes that require the establishment of cell polarity, such as pseudohyphal growth \((p < 1.3 \times 10^{-14})\), conjugation \((p < 2.1 \times 10^{-3})\), cell budding \((p < 5.5 \times 10^{-7})\) and cytokinesis \((p < 2.4 \times 10^{-6})\). Thus a significant fraction of the genes identified in systematic polarity screens (26%) have well-characterized roles in cellular polarization.

We anticipated that our analysis would identify a set of core polarity genes required in all polarity assays. Indeed, genes encoding polarisome components (Bni1, Spa2 and Pea2) and actin cytoskeleton components (Sla1, Tpm1, Sac6, Abp1 and Rvs161) were identified in seven or more screens. We had further predicted that specific biological processes would be enriched depending on the way cell polarity was queried. To test this idea, we defined three general categories in which to bin the published datasets: (i) type of polarized growth (analysis of budding/cytokinesis, filamentous growth or mating); (ii) type of mutation or sensitized background (deletion, overexpression or chemical treatment); and (iii) phenotypic readout (colony size, colony morphology, single-cell analysis or reporter detection) (see electronic supplementary material, table S2). Surprisingly, the same subset of GO Slim functional categories was enriched irrespective of categorization. For example, OST3 (which encodes a subunit of the oligosaccharyltransferase complex important for \(N\)-glycosylation) was identified in a variety of phenotypic

Figure 5. Biological processes enriched in genomic polarity screens. A network diagram was created with Cytoscape and BINGO to visualize biological GO Slim processes that are enriched in a set of 35 genome-wide genetic polarity screens \((n = 485)\).
transcription (p(pingly, while the GI profiles of most mitochondrial genes are
and was identified in 11 of 35 polarity datasets [81]. Interest-
ILM1
on their correlation in global GI profiles [47] but appear fre-
ture. Some biological processes, for instance mitochondrion
amount of variance in several polarity-based phenotypes [122].
phenotypic robustness, and which when deleted cause a high
of 502 identified capacitors), gene products that contribute to
integrity, including
membrane organization (p


role of
an alternative model for the involvement of mitochondrial maintenance
is also implicated
of cell polarity. Of particular note is AIM44, whose product
localizes to the bud neck [35]; a strain deleted for AIM44
is highly sensitive to numerous chemicals affecting polarity pro-
cesses, including papuamide B, latrunculin, caspofungin and
inhibits cellular polarization through the action of the
mitochondrial retrograde signalling pathway which relays
alterations in mitochondrial function to the nucleus, leading
to specific adaptive changes in nuclear gene expression [82].
Our analysis of published cell polarity screens also high-
lighted genes of unknown function that appeared in several
screens and may represent previously unappreciated regulators
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4. Perspectives and future directions

The study of cell polarity in yeast has been dramatically
enhanced by the application of high-throughput approaches,
which can provide an unbiased view of conserved biological
processes. So far, systematic genetic screens have largely
focused on the analysis of single and double deletion mutants
using simple phenotypic read-outs, for example colony size,
which has provided biologically rich information. In the next
few years, cell polarity research is likely to be advanced on
several fronts. First, yeast researchers are now poised to shift
towards large-scale analysis of higher order combinations of
genetic lesions, including triple mutant analysis, and combi-
ations of genetic and chemical perturbations. Higher order
multiplexing of mutant strains is now technically feasible
owing to recent adaptations of pooled competitive growth
assays [58]. Also, most genome-wide screens to date have
used deletion alleles of non-essential genes and mechanisti-
cally uncharacterized conditional alleles of essential genes
[31–34]. Many cell polarity components are essential or have
multiple roles, and their analysis demands a more sophisti-
cated approach. High-throughput methods for generation of
gene mutations [31,125–127] mean it is now feasible to con-
struct strain libraries carrying point mutations in all yeast
genomes, as opposed to deletion or other loss-of-function alleles.
It is clear that SGA-based screens with strains carrying differ-
ent mutations in the same gene provide unique biological
information [32,62,128,129]. Thus, GI analysis with strains car-
rying specific mutations in cell polarity genes promises to lead
researchers into uncharted genetic territory.

Second, advances in automated image acquisition and
analysis for assessment of cell polarity phenotypes in sensi-
tized genetic or conditional backgrounds mean that image-
based screens should move to the forefront of cell polarity
research in yeast [130]. To date, image-based screens using the yeast deletion and other arrayed collections have collected static images in a single focal plane [53,90–98]. However, cell polarity is highly dynamic, and future analyses will involve the high-throughput acquisition of three- and four-dimensional spatial and temporal image sequences, using multiple fluorescent markers to highlight numerous compartments simultaneously. Until recently, the huge amount of data generated by high-dimensional screens has made analysis time-consuming and labour-intensive. However, new algorithms for data extraction and expansions of readily accessible software are making this type of screening more practical for yeast laboratories [131–133], and automated image-based analysis of cell polarity phenotypes should become routine. This is important because visual analysis of complex phenotypes is highly subjective and qualitative, while computational extraction of cellular features and subsequent statistical analysis provides an essential foundation for quantitative biology. Finally, proteomics approaches are also being expanded to take into account the dynamic nature of cellular processes, defining abundance, localization, protein–protein interactions and post-translational modification for the yeast proteome, as well as changes in the proteome in response to different growth conditions [116,117,134]. In the future, using techniques that allow quantification of absolute protein levels at the proteome level, for example stable isotope labelling by amino acids in cell culture (SILAC) [135,136], researchers will be able to accurately assign protein abundance and stoichiometry of cell polarity complexes in specific conditions, for instance the phases of the cell cycle. Computational and mathematical modelling of the cell polarity machinery also provides new perspectives in understanding cell polarity. Some models are fairly linear and are based on positive feedback and global inhibition [137,138], whereas others are more complex and aim to directly address molecular interactions underlying cellular polarization [139,140]. Generally, these models rely on the application and interpretation of our current knowledge of specific targeting sequences and domains of proteins and have already been successfully applied to identify candidate genes, whose roles in polarity have then been assessed experimentally [141]. For example, recent work revealed that multiple clusters of polarity landmarks are formed in budding yeast cells and that the bud site is selected via competition between these sites followed by negative feedback to dismantle all clusters but one [142]. Interestingly, this observation predicts oscillatory polarization, as the positive feedback loop created by the polarity clusters is rapidly antagonized by the negative feedback loop, and hints that this system could be adapted to polarize several axes to produce the complex morphologies observed in higher eukaryotes. The integration of genome-wide data with the predictive power of computational modelling provides a unique way to survey previously unexplored areas of the complete cellular network. Very few groups have ventured into the field of mathematical modelling at a genome-wide level, but the recent success of Chau et al. [141] in modelling all possible feedback loops capable of polarizing the cell highlights the potential for future research in this area. In summary, the budding yeast continues to provide a robust experimental platform to study gene function and malfunction, and a guide for studying and interpreting biological pathways in more complicated systems. Indeed, hypotheses generated in modern applications of yeast systems biology have contributed numerous valuable insights to the broad range of cellular activities that underlie the core biological process of polarity. More than 20 years ago, studies in S. cerevisiae pioneered the identification of the first major cell polarity regulators that are responsible for orienting cell growth. More recent work using high-throughput functional genomics approaches has begun to make sense of how molecular interactions between key polarity determinants are organized within cells and has provided new insight into how polarity is generated and maintained. Ultimately, these types of studies aim to approach the goal of a complete description of the functions and interactions of all the molecular components of a basic eukaryotic cell.

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