sibility caused by genetic factors and drug toxicity. Recently, transgenic mice were produced by retroviral transduction of male germ line stem cells (32). However, the efficiency and the definition of integration of exogenous genes into the genome remained to be improved. It is very difficult to transfer genes into primary cultures of spermatogonia and impossible to select them with drugs in vitro. Use of the spermatogonial cell line may resolve current challenges with primary cultures of isolated spermatogonia and may greatly increase the success and efficiency of generating transgenic mice.

References and Notes
11. A 3441–base pair (bp) EcoR I/EcoR I fragment carrying 3200 bp of full-length murine telomerase reverse transcriptase (mTERT) cDNA was obtained from pCR188 and subcloned into EcoRI site in a retrovirus vector pLXSN (Clonetech). The orientation for expressing sense mTERT by 5′-LTR was checked by digestion with Xho I. Expression of Neo R was driven by SV40 early promoter. Viruses were harvested from supernatants of transfected Phoenix packaging cells.
15. Total RNA was isolated and cDNA synthesis was performed with random hexamers. RT-PCR amplification of rat protamine-2 was 5′-GAGCCTGAAAGGATGAAAGG-3′ and 5′-GGTTCTCATTGTTGTCGGCT-3′ (94°C for 35 s, 55°C for 30 s, and 72°C for 45 s) for 30 cycles. The size of the RT-PCR product was 207 bp. Primers for RT-PCR amplification of protamine-2 were 5′-GACGCGTGAGAGGACTATGG-3′ (94°C for 30 s, 58°C for 30 s, and 72°C for 45 s for 35 cycles). The product was a 282-bp DNA fragment.
21. Single-letter abbreviations for amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
25. Acfc3-EFGP-pDNA3.1/Zeo (-) was created by subcloning the 3200 bp Hind III fragment of Acfc3-EFGP (containing acrosin promoter, the fused peptide MEVRLTPVAVLVAVSLSV-KDN-TGG-EFGP [22], and bovine growth hormone polyadenylate from pUC19/Acr3-EFGP) into pCDNA3.1/Zeo (-).
28. X. Meng et al., Cancer Res. 61, 3267 (2001).
33. We thank M. Okabe for pUC19/Acr3-EFGP, P. Moens for antibody to SCP3, and R. A. DePinho for pGMR188. Supported in part by NIH grant HD-36483.

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Systematic Identification of Pathways That Coupling Cell Growth and Division in Yeast

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Size homeostasis in budding yeast requires that cells grow to a critical size before commitment to division in the late prereplicative growth phase of the cell cycle, an event termed Start. We determined cell size distributions for the complete set of ~6000 Saccharomyces cerevisiae gene deletion strains and identified ~500 abnormally small (whi) or large (gee) mutants. Genetic analysis revealed a complex network of newly found factors that govern critical cell size at Start, the most potent of which were Sfp1, Sch9, Cdc1, Prs1, and Whi5. Ribosome biogenesis is intimately linked to cell size through Sfp1, a transcription factor that controls the expression of at least 60 genes implicated in ribosome assembly. Cell growth and division appear to be coupled by multiple conserved mechanisms.

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ing cultures (24–26). A wide range of size distributions was observed: The smallest strains, such as sfp1Δ and sch9Δ, had a median cell volume ~40% smaller than wild-type cells, and the largest strains, such as cdc10Δ, had a median cell volume >70% larger than wild-type cells (Fig. 1A). We clustered the size distributions with standard algorithms and represented each as a one-dimensional color plot (26–29). Because cell size distributions reflect many parameters, clustering of such distributions can reveal genes with shared functions (fig. S1). This clustering also separated small (whi) and large (lge) cell size haploid deletion mutants from the majority of other viable mutants, whose size distributions were indistinguishable from wild-type controls (Fig. 1B). We classified the largest 5% of deletion strains as Lge and the smallest 5% as Whi (26).

The functions of genes that affect cell size fell into a number of distinct categories. All of the known Start regulators with pronounced cell size defects were isolated as lge (swi4Δ, swi6Δ, cln3Δ, and bck2Δ) or whi (whi3Δ) deletion strains. Debitillation of any process important for cell cycle progression but not growth should, in principle, lead to an increase in cell size (4). Indeed, the predominant class of lge mutants corresponded to genes involved in cell cycle progression (fig. S2). Large cell size also resulted from perturbation of the actin cytoskeleton, secretory pathways, translation components, and global regulators of RNA Pol II transcription, all of which can directly or indirectly affect cell cycle progression. The majority of whi strains lacked components of the ribosome or the mitochondrial respiratory apparatus (fig. S2) (26). The small cell size of these mutants is probably a consequence of slow growth rate, which causes cells to accumulate in G1 phase. Gene deletions that attenuated translation, ribosome biogenesis, and glucose signaling also diminished cell size, as did 33 candidate WHI genes of unknown function.

Given that some essential genes might regulate cell size and that Start regulators are often dosage sensitive (15, 16), we sized the complete set of 1142 diploid strains heterozygous for an essential gene deletion (fig. S1). Heterozygous diploid strains were increased up to ~40% (rpt2Δ/RPT2) or decreased down to ~15% (cdc24Δ/CDC24) in median cell size. Haploinsufficient lge mutants corresponded to components of the 26S proteasome, components of a γ-tubulin complex, genes involved in ribosome biogenesis, and several other processes, whereas haploinsufficient whi mutants corresponded to factors involved in ribosome biogenesis, nucleolar function, and ribosomal subunits (fig. S2).

The ~500 haploid deletion strains with size distributions distinct from those of wild-type cells required additional tests to reveal the genes with direct roles in cell size control at Start. To determine which of the 249 lge strains are perturbed for Start progression, we tested for synthetic lethal interactions with known activators of Start. For example, CLN3 and BCK2 act in parallel pathways such that cln3Δ bck2Δ double mutants are not viable due to G1 arrest (21), whereas cln3Δ swi6Δ and bck2Δ swi6Δ double mutants have growth defects in progression through Start (21, 23). We subjected the entire set of 249 lge strains to synthetic genetic array (SGA) analysis to systematically screen for genetic interactions with cln3Δ, bck2Δ, and swi6Δ (26, 30). SGA detected 23 candidate genetic interactions, 12 of which were confirmed by direct tetrad analysis (fig. 2A). In addition to known interactions, bck2Δ and cln3Δ exhibited a synthetic growth defect only with deletion of SST2, a negative regulator of the mating pathway (31). In contrast, swi6Δ

![Fig. 1. A systematic survey of cell size mutants in S. cerevisiae. (A) Cell size distributions for a wild-type (WT) strain as compared to two of the smallest deletion strains, sfp1Δ and sch9Δ, as well as one of the largest strains, cdc10Δ. Distributions are visualized as colored strips, in which all size bins above an arbitrary number % threshold of 0.5% (dotted line) are colored with an intensity proportional to number %. (B) Hierarchical clustering of cell size distributions of 4812 viable strains with deletions in genes whose products form part of Start. For example, CLN3 and BCK2 revealed a network of Start activators. Confirmed synthetic genetic interactions (12) between lge strains (cdc73Δ, sst2Δ, sec66Δ, rps21bΔ, ypl055cΔ, cla4Δ, bem1Δ, bem2Δ, and bem4Δ) and swi4Δ, cln3Δ, and bck2Δ are represented by lines. Deletion strains are represented by colored nodes: G1/S transcription (black), bud emergence (blue), pheromone signaling (green), or other processes (red). Synthetic genetic interactions (Fig. 4A) were also discovered between several whi mutants (gpa2Δ, sch9Δ, sfp1Δ, cdh1Δ, and whi5Δ) and swi4Δ (pink nodes). (B) The Whi phenotype of 25 deletion strains is not solely attributable to slow growth rate. A plot of doubling time versus cell size for two WT strains and 25 viable deletion strains (solid squares) was used to establish a baseline correlation between doubling time and small cell size (dotted line). Another 61 whi strains (gray circles) are plotted, including 10 whi strains with deletions in genes whose products form part of a nucleolar network (open triangles). The 25 strains that partially uncouple growth from division fall on or below the baseline fitted to the smallest ribosomal gene deletion strains. (C) WHIS, CDH1, and SFP1 are negative regulators of Start. Early G1-phase daughter cells were isolated by centrifugal elutriation, released into glucose medium, and monitored for bud emergence. In plots of bud index versus modal cell size, Start was defined as 25% budded (19).](http://www.sciencemag.org/content/sci/297/5578/296.full.html)
exhibited synthetic genetic interactions with ige strains defective in bud emergence (cln3Δ, bem1Δ, bem2Δ, and bni4Δ). Start-specific transcription (cln3Δ, bck2Δ, and cdc73Δ), a 40S ribosome component (prs3Δ/H9004), and a secretory pathway component (sec62Δ). Synthetic lethality was also observed with a deletion of an uncharacterized gene, YPL055C, a candidate activator of Start that we named LGE1.

To identify potential repressors of Start, we characterized 61 haploid whi strains that were not directly compromised in ribosomal or respiratory function. Deletion of genes that regulate Start would be expected to have a greater effect on cell size than could be explained by any associated effects on the rate of cell growth (4, 32). Doubling time was plotted against both median and daughter cell size for the 61 whi mutants and, as controls, 2 wild-type and 18 ribosomal gene deletion strains (Fig. 2B). The distribution of doubling time versus cell size for the wild-type strains and the smallest of the ribosomal gene deletion strains established a baseline correlation between growth rate and cell size. Potential negative regulators of Start, represented by 25 whi strains, fell on or below this baseline. Of these, only a few have been previously implicated in cell cycle control: WHI3 encodes a negative regulator of CLN3 (18, 20), and CDH1 encodes an activator of the anaphase promoting complex/cyclosome (APC/C), which maintains G1 phase in fission yeast and flies (33). Two of the smallest strains isolated in the initial screen, sfp1Δ and sch9Δ, fell well below the baseline (Fig. 2B). SFP1 encodes a predicted zinc finger transcription factor with a suggested role in the response to DNA damage and the G1-to-S transition (M phase transition (34)). The small cell size of sfp1Δ strains had been previously noted but not characterized (34). A fortuitous hypomorphic allele of SFP1, created by addition of an epitope tag sfp1- myc13, conferred a small cell size with only a marginal increase in doubling time (Fig. 2B). Sch9 is 49% identical to human Akt1 in the COOH-terminal kinase domain and has been implicated in the control of longevity and stress resistance, as have its metazoan counterparts (14, 35). SCH9 is also a haploinsufficient whi mutant, suggesting it may be a dosage-dependent repressor of Start (see Fig. 3C).

We tested the panel of 25 whi mutants for Start-related phenotypes (Table 1). Three mutants, whi3Δ, yor085wΔ, and ykr020wΔ, were resistant to mating pheromone, a phenotype associated with premature traversal of Start (16, 17, 20); therefore, we renamed YOR085W and YKR020W as WHI5 and WHI6, respectively. To confirm the effects on Start of three potential whi mutations, we directly measured their critical cell size, as operationally defined by the cell volume at which bud emergence occurs. Early G1-phase daughter cells from wild-type, whi5Δ, cdh1Δ, and sfp1Δ cultures were isolated by centrifugal elutriation (19, 26), and progression through Start was monitored by bud index (Fig. 2C). A whi5Δ strain passed Start after growing to just 15 fl, half the critical cell size of congenic wild-type cells, which budded at 32 fl. Reductions in critical cell size were also observed in sfp1Δ and cdh1Δ strains, which budded at 23 fl and 27 fl, respectively. Each whi strain tested, the onset of RNR1 transcription was accelerated as compared to wild-type strains (36). Therefore, WHI5, CDH1, and SFP1 encode novel repressors of Start that likely act upstream of SBF and MBF.

To elucidate connections between newly found Start repressors and known pathways, we performed 58 pairwise matings of 12 of the 25 whi mutants in Table 1 to wild-type, cln3Δ, bck2Δ, swi4Δ, and cdh1Δ strains (26). Seven whi mutants showed only additive effects on cell size in combination with the known Start regulators. Epistatic size interactions were observed with whi5Δ, prs3Δ, and bck2Δ, and more complicated interactions were observed with whi5Δ and sch9Δ (Fig. 3A). The size distribution of the whi5Δ swi4Δ double mutant was nearly identical to that of swi4Δ, again suggesting that Whi5 acts upstream of Swi4 (Fig. 3B). The small cell size caused by prs3Δ was almost completely unaffected by additional cln3Δ, bck2Δ, or swi4Δ mutations that alone cause a pronounced large cell size phenotype (Fig. 3B) (36). Thus, in the absence of PRS3, the canonical regulators CLN3, BCK2, and SWI4 have almost no effect on the timing of Start. PRS3 encodes one of five ribose-phosphate pyrophosphokinases in yeast and may be required for cell cycle arrest in response to nutrient deprivation (37). Lastly, the size of bck2Δ cdh1Δ double mutants was indistinguishable from that of the cdh1Δ single mutant, suggesting that CDH1 may act downstream of PTK2 in the same pathway (Fig. 3B). These crosses also uncovered a number of synthetic genetic interactions between some whi mutants (Fig. 2A), such as between sfp1Δ and sch9Δ, which may therefore converge on the same essential process.

Given the paucity of epistatic interactions between established Start pathways and our newly identified cell size regulators, it seems likely that uncharacterized pathways control the critical cell size threshold. A major pathway appears to involve aspects of ribosome biogenesis. Deletion of any of five nonessential genes implicated in ribosome biogenesis, RPA14,
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*RPA49, SSF1, SKY1, and TOM1*, caused substantial decreases in cell size without a proportionate decrease in growth rate (triangles in Fig. 2B, Table 1). Heterozygous deletion in any of 10 essential genes involved in ribosome biogenesis caused decreases in cell size but with less severe increases in doubling time than those caused by heterozygous deletions in essential subunits of the ribosome (table S2, Fig. 3C). Thus, the reduction in cell size caused by defects in ribosomal biogenesis appears not to be solely a consequence of reduced growth rate.

Expression of 11 of 15 of the size control genes implicated in ribosome biogenesis depended on Sfp1. Not only was the *sfp1Δ* strain one of the smallest *whi* mutants recovered, overexpression of *SFP1* resulted in very slow growth and a Lge phenotype (36). To determine the direct transcriptional targets of Sfp1, an *sfp1Δ::GALI-SFP1* strain was shifted from raffinose to galactose medium, and the consequences of restoring *SFP1* expression were monitored by DNA microarray analysis over time (t). (26). Shortly after *SFP1* induction, expression of 116 genes was evident; of these, 73 fell into the discrete functional categories of nucleotide biosynthesis, transfer RNA synthetases, ribosome biogenesis, ribosomal RNA (rRNA) transcription, and translation initiation and elongation (Fig. 4A, t = 30 min). Of the remaining 43 genes, 21 encoded proteins that form multiple interactions within a nuclear protein network. After induction of the initial gene set, nearly all of the genes encoding ribosomal proteins (*RP*s) were collectively induced (Fig. 4A, t = 50 min). All of the gene clusters induced by *SFP1* were repressed in an *sfp1Δ* strain (Fig. 4A). Many genes implicated in ribosome biogenesis are transcriptionally co-regulated, probably through two promoter elements termed RRPE and PAC (38, 39). The set of *Sfp1*-regulated early genes was enriched for RRPE and PAC elements, which thus represent candidate binding sites for Sfp1 (Fig. 4A). *RP* gene promoters did not contain RRPE and PAC elements, whereas *Sfp1*-regulated early gene promoters did not contain binding sites for Rap1 (40), a transcriptional

### Table 1. Characterization of *whi* mutants. Strains at or below the growth rate–cell size baseline as defined in Fig. 2B are listed in order of increasing size; ± 1 SD is reported for each parameter. All strains listed here showed tight linkage between *kanR*-marked gene deletion and the Whi phenotype. Homologs have 25% amino acid identity over more than half the protein, except for SPC22G7.02 and importin 13, which only have ~20% identity. R, resistance; S, sensitivity to mating pheromone α-factor, relative to the response of the wild-type strain (WT) (26). ORF overlaps between *YGR064W* and *SFP1* and between *YNL227C, YNL226W, and YNL228W* preclude definitive assignment of the Whi phenotype. NA, not applicable.

<table>
<thead>
<tr>
<th>Deleted gene</th>
<th>Doubling time (min)</th>
<th>Haploid cell size (fl)</th>
<th>1N DNA content (%)</th>
<th>% budded</th>
<th>Homolog(s)</th>
<th>Known role</th>
<th>Human</th>
</tr>
</thead>
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<tr>
<td><strong>WT</strong></td>
<td>20 ± 12</td>
<td>25 ± 1</td>
<td>73 ± 0 24 ± 4</td>
<td>S</td>
<td></td>
<td></td>
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<tr>
<td><strong>SCH9</strong></td>
<td>153 ± 2</td>
<td>28 ± 1</td>
<td>64 ± 3 43 ± 2</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PRS3</strong></td>
<td>135 ± 3</td>
<td>30 ± 1</td>
<td>48 ± 2 65 ± 2</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>WHI3</strong></td>
<td>108 ± 3</td>
<td>31 ± 1</td>
<td>42 ± 2 57 ± 7</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>WHI5</strong></td>
<td>88 ± 2</td>
<td>32 ± 1</td>
<td>34 ± 4 68 ± 7</td>
<td>S</td>
<td></td>
<td></td>
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<tr>
<td><strong>PA49</strong></td>
<td>137 ± 8</td>
<td>33 ± 1</td>
<td>57 ± 6 44 ± 7</td>
<td>S</td>
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<tr>
<td><strong>YNL227C</strong></td>
<td>124 ± 9</td>
<td>34 ± 1</td>
<td>50 ± 2 42 ± 5</td>
<td>S</td>
<td></td>
<td></td>
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<tr>
<td><strong>YNL226W</strong></td>
<td>126 ± 11</td>
<td>44 ± 1</td>
<td>52 ± 4 45 ± 4</td>
<td>S</td>
<td></td>
<td></td>
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<tr>
<td><strong>PHOS</strong></td>
<td>125 ± 3</td>
<td>41 ± 1</td>
<td>46 ± 2 49 ± 1</td>
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<tr>
<td><strong>HXX2</strong></td>
<td>86 ± 4</td>
<td>34 ± 1</td>
<td>51 ± 6 59 ± 3</td>
<td>S</td>
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<tr>
<td><strong>YHR034C</strong></td>
<td>111 ± 7</td>
<td>38 ± 1</td>
<td>61 ± 1 38 ± 6</td>
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<tr>
<td><strong>SKY1</strong></td>
<td>97 ± 4</td>
<td>47 ± 1</td>
<td>52 ± 2 47 ± 7</td>
<td>S</td>
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<tr>
<td><strong>KAP122</strong></td>
<td>91 ± 1</td>
<td>53 ± 1</td>
<td>52 ± 4 53 ± 6</td>
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<td><strong>SSF1</strong></td>
<td>107 ± 8</td>
<td>41 ± 1</td>
<td>53 ± 1 41 ± 6</td>
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<tr>
<td><strong>RPA14</strong></td>
<td>97 ± 2</td>
<td>51 ± 1</td>
<td>48 ± 2 61 ± 5</td>
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<tr>
<td><strong>YGR111W</strong></td>
<td>103 ± 6</td>
<td>36 ± 1</td>
<td>46 ± 1 56 ± 2</td>
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<tr>
<td><strong>YCR066W</strong></td>
<td>91 ± 1</td>
<td>57 ± 1</td>
<td>45 ± 2 57 ± 10</td>
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<td><strong>PTK2</strong></td>
<td>88 ± 3</td>
<td>36 ± 1</td>
<td>46 ± 1 45 ± 2</td>
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<tr>
<td><strong>TOM1</strong></td>
<td>99 ± 0</td>
<td>37 ± 1</td>
<td>44 ± 2 57 ± 2</td>
<td>S</td>
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<td><strong>GRA2</strong></td>
<td>91 ± 6</td>
<td>37 ± 0</td>
<td>55 ± 7 53 ± 12</td>
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<tr>
<td><strong>KEL1</strong></td>
<td>87 ± 4</td>
<td>37 ± 1</td>
<td>41 ± 2 69 ± 1</td>
<td>S</td>
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<td><strong>SWE1</strong></td>
<td>91 ± 2</td>
<td>51 ± 0</td>
<td>49 ± 1 50 ± 1</td>
<td>S</td>
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</tr>
<tr>
<td><strong>YGR064W</strong></td>
<td>91 ± 1</td>
<td>39 ± 1</td>
<td>41 ± 2 69 ± 1</td>
<td>S</td>
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<tr>
<td><strong>CDH1</strong></td>
<td>115 ± 4</td>
<td>64 ± 1</td>
<td>35 ± 3 64 ± 6</td>
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<tr>
<td><strong>WHI6</strong></td>
<td>102 ± 5</td>
<td>43 ± 3</td>
<td>50 ± 2 52 ± 3</td>
<td>S</td>
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<tr>
<td><strong>WT</strong></td>
<td>87 ± 6</td>
<td>42 ± 2</td>
<td>44 ± 1 59 ± 5</td>
<td>S</td>
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*Proteins that belong to members of highly conserved families with multiple *S. pombe* and/or human homologs. †Strains subjected to epistasis analysis.
activate of *RP* genes (Fig. 4A). On the basis of the kinetics of gene induction, one or more Sfp1-regulated genes may cue Rap1 to express *RP* genes in a transcriptional cascade that ultimately controls both critical cell size and ribosome biogenesis.

The nucleolus is the site of ribosome construction, in which RNA is transcribed, processed, and assembled into ribosomes (41). Ribosome assembly is thought to be mediated by large macromolecular factories; as an organelle, the nucleolus is defined not by membranes but by protein-RNA, protein-DNA, and protein-protein interactions (41). Of the 15 factors implicated in cell size control and ribosome biogenesis, 13 are components of a large network highly enriched in nucleolar proteins, as defined by large-scale protein interaction data sets (26). This nucleolar network may contain up to 250 proteins, depending on how the network is defined. We assembled a subnetwork containing the 13 cell size control proteins and 106 other highly connected proteins (Fig. 4B). Of these 119 factors, 59 have been implicated in ribosome biogenesis (31), 46 have been localized to the yeast nucleolus (31), and 39 are homologs of proteins found in purified human nucleoli (42). About half of this network was controlled at the level of Sfp1-dependent transcription (red dots, Fig. 4B).

The above synthetic lethal interactions, epistatic relations, and DNA microarray data identify and partially order pathways that establish the critical cell size threshold at Start (Fig. 4C). The conventional pathway for activation of Start includes Cln3-Cdc28 and Bck2, which trigger the G1-S gene expression program by activating the SBF and MBF transcription factors. Our genomewide analysis suggests that up to 40 previously unidentified components also control Start, many of which are implicated in ribosome biogenesis (Fig. 4C). Of the newly identified Start regulators that we have studied, *WHI5, CDH1*, and *SFP1* appear to act upstream of SBF and MBF but not through Cln3 and Bck2 (Figs. 2C and 3A). Whi5 does not have similarity to other known proteins, but it is expressed in a G1-periodic manner (22), consistent with a role at Start. On the basis of its known biochemical role as a substrate recruitment factor for the APC/C (33), Cdhl may target one or more activators of Start for ubiquitin-dependent proteolysis. Amongst the factors implicated in ribosome biogenesis, Sfp1 is the most potent repressor of Start, presumably because it controls the expression of numerous genes that influence cell size.

The partial uncoupling of growth from division by specific mutations in ribosome biogenesis pathways suggests that the critical cell size threshold is not set simply as a passive downstream readout of protein synthetic rate (43). Instead, commitment to division may be dynamically linked to signals that stimulate ribosome biogenesis, which is the predominant biosynthetic activity of a growing yeast cell (44). That is, the cell may anticipate changes in its protein synthetic rate by adjusting the critical cell size threshold before any actual change in ribosome content. This interpretation is consistent with the observation that the critical cell size threshold increases in nutrient-rich conditions, as do the rates of ribosome biogenesis and protein synthesis (6, 44). Although the nature of the repressive influence of ribosome biogenesis on Start remains to be determined, the interplay between ribosome assembly and cell cycle progression appears to be a conserved feature of eukaryotic cell division (14, 45, 46). In the absence of S6K, which stimulates growth

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**Fig. 4.** Sfp1 controls expression of a nucleolar network that is enriched in cell size control genes. (A) DNA microarray analysis revealed Sfp1-dependent expression of 94 genes in the indicated functional classes. Congenic wild-type and sfp1::GAL1-SFP1 strains grown in rich medium containing 2% raffinose were induced with 2% galactose and were harvested at the indicated time points (min). Expression ratios relative to the initial raffinose culture are shown for each time point. Red indicates >1.5-fold increase (induction), green indicates <1.5-fold decrease (repression), and gray signifies that no reliable measurement was obtained. The transcriptional profile of cells lacking Sfp1 was determined by comparing raffinose-grown sfp1::GAL1-SFP1 versus WT strains (GAL1-SFP1 column) or glucose-grown sfp1::Δ::kanR versus WT strains (Δ::column). Genes induced early in the sfp1::GAL1-SFP1 time course contained strong matches to upstream RRPE (light blue) and PAC (dark blue) promoter elements but not RAP1 elements, which activate *RP* gene transcription (purple). The number of genes in each functional class is indicated in parentheses. For an enlarged version of Fig. 4A, see fig S3. (B) Thirteen cell size control genes implicated in ribosome biogenesis are embedded in a nucleolar protein interaction network. Interactions between proteins encoded by 13 cell size control genes (squares, 10 haploinsufficient *whi* strains from Fig. 3C; diamonds, 3 *whi* strains Table 1) and 106 of the most highly interconnected proteins, as determined from large-scale coimmunoprecipitation data, are shown in a binary representation (26). Gene names are to the lower right of each node. Transcriptional targets of Sfp1 are indicated by red circles. (C) Summary of cell size control genes that act at Start. Epistatic interactions were assumed to reflect linear pathways, but parallel pathways are also formally possible. Dashed lines indicate uncertain interactions. Genes that were not ordered are not shown.
by activating the translation of ribosomal components, fly cells become abnormally small (14, 45). Pathways that control critical cell size at Start in budding yeast may provide further insight into mechanisms that couple growth and division in higher organisms.

References and Notes

25. Cultures were grown in rich media containing 2% glucose at 30°C to 0.3 to 10^6 cells/ml, a range in which wild-type size distributions do not vary. Cell clumps were dispersed with a sonicator, and size distributions were obtained with a Coulter Channelizer ZZ (Beckman-Coulter).

Materials and methods are available as supporting online material on Science online.


The elucidation of underlying biological mechanisms that contribute to individual differences in both normal and abnormal behavior remains a crucial and largely unmet challenge. Advances in both molecular genetics and psychopathology, especially anxiety traits, but the predictive value of this genotype against these complex behaviors has been inconsistent. Serotonin [5-hydroxytryptamine, (5-HT)] function influences normal fear as well as pathological anxiety, behaviors critically dependent on the amygdala in animal models and in clinical studies. We now report that individuals with one or two copies of the short allele of the serotonin transporter (5-HTT) promoter polymorphism, which has been associated with reduced 5-HTT expression and function and increased fear and anxiety-related behaviors, exhibit greater amygdala neuronal activity, as assessed by BOLD functional magnetic resonance imaging, in response to fearful stimuli compared with individuals homozygous for the long allele. These results demonstrate genetically driven variation in the response of brain regions underlying human emotional behavior and suggest that differential excitability of the amygdala to emotional stimuli may contribute to the increased fear and anxiety typically associated with the short SLC6A4 allele.

Serotonin Transporter Genetic Variation and the Response of the Human Amygdala

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A functional polymorphism in the promoter region of the human serotonin transporter gene (SLC6A4) has been associated with several dimensions of neuroticism and psychopathology, especially anxiety traits, but the predictive value of this genotype against these complex behaviors has been inconsistent. Serotonin [5-hydroxytryptamine, (5-HT)] function influences normal fear as well as pathological anxiety, behaviors critically dependent on the amygdala in animal models and in clinical studies. We now report that individuals with one or two copies of the short allele of the serotonin transporter (5-HTT) promoter polymorphism, which has been associated with reduced 5-HTT expression and function and increased fear and anxiety-related behaviors, exhibit greater amygdala neuronal activity, as assessed by BOLD functional magnetic resonance imaging, in response to fearful stimuli compared with individuals homozygous for the long allele. These results demonstrate genetically driven variation in the response of brain regions underlying human emotional behavior and suggest that differential excitability of the amygdala to emotional stimuli may contribute to the increased fear and anxiety typically associated with the short SLC6A4 allele.

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