Arabidopsis TCP20 links regulation of growth and cell division control pathways

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During postembryonic plant development, cell division is coupled to cell growth. There is a stringent requirement to couple these processes in shoot and root meristems. As cells pass through meristems, they transit through zones with high rates of cell growth and proliferation during organogenesis. This transition implies a need for coordinate regulation of genes underpinning these two fundamental cell functions. Here, we report a mechanism for coregulation of cell division control genes and cell growth effectors. We identified a GCCCR motif necessary and sufficient for high-level cyclin *CYCB1;1* expression at G_2/M. This motif is overrepresented in many ribosomal protein gene promoters and is required for high-level expression of the S27 and L24 ribosomal subunit genes we examined. $p33^{TCP20}$, encoded by the *Arabidopsis TCP20* gene, binds to the GCCCR element in the promoters of cyclin *CYCB1;1* and ribosomal protein genes *in vitro* and *in vivo*. We propose a model in which organ growth rates, and possibly shape in aerial organs, are regulated by the balance of positively and negatively acting teosinte-branched, cycloidea, PCNA factor (TCP) genes in the distal meristem boundary zone where cells become mitotically quiescent before expansion and differentiation.

cyclin B1;1 | growth control | teosinte-branched, cycloidea, PCNA factor genes

Enhanced expression of mitotic cyclin *CYCB1;1* in transgenic *Arabidopsis* or of the D-type cyclin *CYCD2;1* in tobacco has yielded plants with accelerated organ growth, without affecting overall developmental control or final organ size in determinate organs (1, 2). These results are paradoxical, because cell proliferation does not proceed without concomitant cell growth.

Plant organ growth is mediated in aggregate by three processes: cell growth, division, and expansion. Although the net outcome of cell growth and cell expansion is cell enlargement, the difference between these two is not semantic because they are driven by different processes. During cell growth, the increase in mass is a consequence of stimulated macromolecular, mostly protein, synthesis required for enhanced metabolism. In meristems and organs, cell growth is necessary to ensure the survival of cells after division and, hence, must be spatially and temporally tightly coupled with proliferation. In contrast, cell expansion predominates in postmitotic cells, accounts for the bulk of the overall size increase in developing organs, and is therefore responsible for most of the growth of the plant body. In cell expansion, mass increase is largely due to osmotically driven water uptake and the initiation of cell enlargement spatio-temporally coincides with the development and expansion of vacuoles. Despite their fundamental importance for plant growth, the genetic mechanisms governing cell growth and size are far less well understood than those regulating division and expansion.

In a shoot or root meristem, cells transit through a succession of developmental zones with different local rates of cell division. Spatial analysis of cell division patterns in meristems has revealed that stem cells, the ultimate source of all shoot and root cells, proliferate very slowly. Their immediate progeny, which form the flanks of the shoot meristem from which lateral organ primordia arise, or the initials in the root that generate the cell types of the root, proliferate slightly faster (3–5). Subsequently, in newly initiated leaf primordia and in the domain distal to the initials in the root, a rapid increase of cell division rates is observed (5–7). We term this the zone of multiplicative divisions. Finally, the rates of cell division gradually decline at the distal end of the root meristem and in a distal–proximal gradient in leaves as cells begin to differentiate (6–10). Analysis of cell morphology in these zones has revealed a gradual size increase as cells progress through these zones (4, 5, 11, 12). Specifically, no size reduction as cells transit into the zone of rapid proliferation was observed in roots (ref. 12 and J. Dubrovsky and P.D., unpublished data).

To maintain approximately constant cell sizes in meristems, the rates of cell division and cell growth must locally be identical; this implies a fundamental requirement for coordinate regulation of cell growth and division in the meristem, specifically in the zone of multiplicative divisions, where proliferation rates change rapidly. Such coordinate regulation is revealed when shoot apices are treated with oryzalin, an inhibitor of mitosis: Cells in young primordia grow more than cells in the central or peripheral zones of the meristem, revealing differential control of growth rates (4). Cell growth requires increased rates of metabolism, mediated by up-regulation of ribosome synthesis and other components involved in protein synthesis (13). Stimulated ribosome biogenesis involves coordinated gene expression mediated by all three types of RNA polymerase; however, coordination of gene expression programs for cell division and cell growth is likely to rely on RNA polymerase II-dependent transcription.

Here, we examine quantitative aspects of *Arabidopsis* mitotic cyclin *CYCB1;1* and ribosomal protein gene L24 and S27 expression and report a DNA sequence motif necessary and sufficient for high-level expression. This motif is overrepresented in promoters of genes that function in ribosome biogenesis. We show that the protein encoded by *Arabidopsis TCP20* specifically binds to the cyclin B1;1 promoter, as well as to several ribosomal protein promoters, thereby revealing mechanistic links between growth and cell cycle control in plants.

Methods

Constructs. We made 5′ deletions of the *CYCB1;1* promoter in pCDG (7) with exonuclease III (14). Constructs were ligated into the pBIB vector (15) to give p351CDG, p205CDG, p143CDG,

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Abbreviations: TCP, teosinte-branched, cycloidea, PCNA factor; ChIP, chromatin immunoprecipitation; RP, ribosomal protein.

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p120CDG, and p60CDG, with the numbers indicating the 5′ deletion endpoint relative to the transcription start site. Constructs expressing *CYCB1;1* were made by replacing a XhoI–SacI fragment from *uid*A with a XhoI–SacI fragment from the *CYCB1;1* cDNA. Modification of candidate binding sites was made by inverse PCR. Gain-of-function constructs were generated on the basis of the −46 35S minimal promoter (16), and contained five or three copies each of the GCCCR or the MSA motif (17), or both, respectively. The minimal promoter, or synthetic promoters were used to express the cyclin-GUS (CGU) cassette in pCDG (7) downstream of the translation start site.

Plant Material and Transformation. *Arabidopsis thaliana* Col-0 was grown at 21°C with a 16-h light/8-h dark cycle. Bright Yellow 2 tobacco (BY2) cells were grown at 27°C in the dark (18). Plants and cell cultures were transformed with *Arabidopsis tumefaciens* GV3101 (19) as described by Clough *et al.* (20) and An (21), respectively. At least 100 calli were pooled for each transgenic BY2 cell culture.

Expression Analysis. For histochemical or fluorometric GUS analysis, tissues were assayed as described (7, 22). Tobacco BY2 cells were synchronized as described (7, 18). RNA was isolated with TRIzol (Invitrogen). RNA (0.5 μg) was reverse-transcribed by using oligo(dT) or gene-specific primers. PCRs (see *Supporting Text*, which is published as supporting information on the PNAS web site, for primer sets) all contained one primer designed to cross an intron and were performed in quadruplicate with the iCycler iQ (Bio-Rad). To normalize for cDNA loading, threshold cycle differences were obtained by subtracting the mean threshold cycle (MTC) for each gene (designated *a*) from the MTC of β-ATPase (23) amplification from the same cDNA sample (designated *b*). Relative amounts were then calculated by subtracting this value from the MTC of the sample designated to be the reference (designated *c*). To represent relative amounts, these logarithms were inverted by using the relationship: relative amount $= 2^{c-(b-a)}$.

***In Vivo* Footprint Analysis.** *In vivo* footprinting was performed by using *Arabidopsis* suspension cells (24) or mature *Arabidopsis* leaves as described (25–27), using separate primer sets for upper and lower strands (see *Supporting Text*).

EMSA. Full-length TCP20 protein was expressed as GST-fusion in *E. coli*, purified by affinity chromatography, and subsequently cleaved from GST. EMSA was conducted with radiolabeled oligonucleotides corresponding to wild-type or mutated GCCCR motifs. Binding reactions were done at room temperature for 20 min in 25 μl containing 20 mM Tris·HCl (pH 8.5), 50 mM KCl, 5 mM MgCl2, 1 mM EDTA, 1 mM DTT, 200 ng/μl BSA, 10% glycerol, 1 μg poly (dI-dC), 1 ng of labeled, probe and 0.1–1 μg of protein. For competition experiments, a 100- to 500-fold molar excess of unlabeled competitors was added 10 min before the labeled probe.

Chromatin Immunoprecipitation (ChIP). Antibodies were raised in sheep against a GST-fusion protein corresponding to amino acids 275–315 of TCP20. Antibodies were affinity purified, and 5-μl aliquots were used for ChIP experiments using extracts from young seedlings (28). See *Supporting Text* for primer sequences. All reactions included primers for the internal control At4. Band intensities were measured with NIH IMAGE version 1.62, and enrichment was calculated as ratio of precipitated versus input DNA after normalization to At4.

Results

Enhanced Expression of *Arabidopsis* CYCB1;1 Stimulates Growth. Increased expression of *Arabidopsis CYCB1;1* under control of the *Arabidopsis CDKA;1* promoter enhances root and shoot growth (1). *CYCB1;1* expression peaks at G_2/M in the cell cycle (1, 7, 29), whereas the *CDKA;1* promoter directs expression at uniform levels throughout the cell cycle (30–33). This raised the possibility that enhanced growth observed in *CDKA;1*::*CYCB1;1* transformed plants was due to an unknown *CYCB1;1* function in the G_1 phase of the cell cycle. To address whether *CYCB1;1* was an effector of growth control pathways at G_2/M, we increased expression of *CYCB1;1* specifically at G_2/M. *Arabidopsis* was transformed with constructs in which *CYCB1;1* was expressed under its own promoter. In lines expressing *CYCB1;1* at high levels (data not shown) due to variation of expression observed in individual transformation events, we observed enhanced shoot (data not shown) and root growth rates (Table 1, which is published as supporting information on the PNAS web site). The cell length of mature cortical cells was very similar in wild-type Col-0 and these transgenic lines (Col-0, 164.1 μm ± 1.96 μm SEM; 11D, 165.6 μm ± 1.57 μm SEM; 26A, 165.5 μm ± 1.72 μm SEM) and, therefore, we concluded that enhanced organ growth was due to increased cell production. Hence, *CYCB1;1* is an effector for growth control at G_2/M. Together with our previous report that *CYCB1;1* expression levels limit growth (1), these results highlight the importance of quantitative control of *CYCB1;1* expression for plant growth.

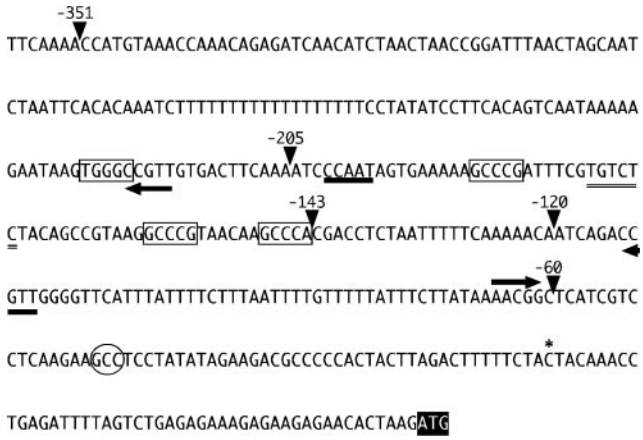

Fig. 1. Proximal *CYCB1;1* promoter. The transcription start site, indicated by an asterisk, was determined by primer extension (data not shown). Endpoints of 5′ deletions are indicated by arrowheads. Possible CCAAT and ARF-binding sites are indicated by thick and double underscore, respectively. GCCCR elements are boxed and MSA elements are indicated by arrows. The hypersensitive footprint is marked with an oval and the lettering of the start codon is inverted.

Qualitative and Quantitative *cis*-Elements in the CYCB1;1 Promoter. To identify the regulatory elements responsible for *CYCB1;1* expression, we used a cyclin–GUS gene fusion (pCDG, with 1.2 kb of 5′ DNA sequence) (7), to generate 5′ promoter deletions and analyzed these in transformed *Arabidopsis* plants and tobacco BY-2 cells. Removal of 5′ promoter sequences up to −351 relative to the transcription start site (Fig. 1) revealed no detectable differences in expression pattern or level, when compared to pCDG (data not shown). Therefore, we focused subsequent analysis on the promoter deletions terminating 351, 205, 143, 120, and 60 bp upstream of the transcription start, respectively (Fig. 1). Histochemical analysis of 5–12 homozygous plant lines for each construct revealed similar levels of expression in plants carrying p351CDG and p205CDG when compared to the full-length promoter. However, further 5′ deletion of promoters to −143, −120, and −60 resulted in a drastic reduction of histochemical staining.

To delineate the promoter elements responsible for regulating

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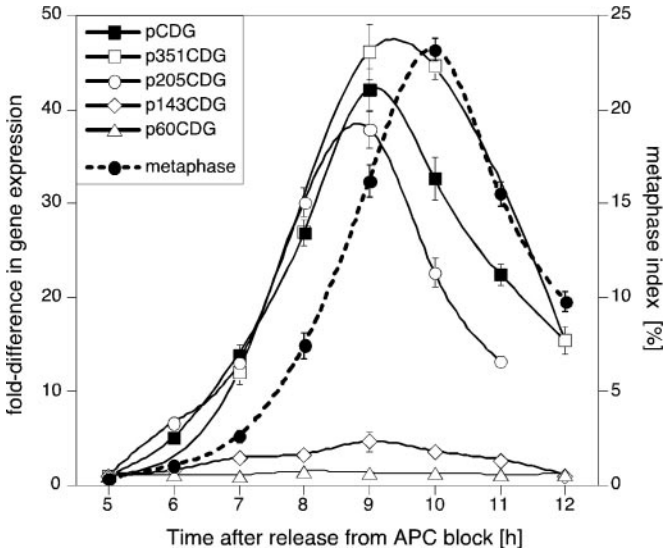

Fig. 2. A major quantitative determinant of *CYCB1;1* expression. BY2 cells were transformed with *CYCB1;1* promoter 5′ deletion constructs directing expression of a chimeric cyclin-GUS (CGU) marker. RNA was isolated from BY2 cells synchronized with Aphidicolin and analyzed by real time RT-PCR. The figure shows gene expression (left axis) and metaphase index (right axis) relative to the values measured 5 h after release from Aphidicolin block. Expression of the CGU reporter in mitotic cells was significantly lower when the DNA sequence between −205 and −143 was removed from the promoter.

CYCB1;1 RNA levels, we analyzed RNA in synchronized BY2 cells transformed with the aforementioned constructs. Cyclin-GUS (CGU) RNA levels were normalized to endogenous β-ATPase mRNA levels, which do not fluctuate during the cell cycle (23). Quantitative analysis in cells transformed with pCDG, p351CDG and p205CDG revealed high-level induction of CGU RNA as cells approached M phase (Fig. 2). In contrast, cells transformed with p143CDG expressed CGU RNA with 8- to 10-fold lower amplitude but still with appropriate cell cycle timing (Fig. 2). Further 5′ deletion of the promoter to −60 led to the loss of M phase induction of CGU expression (Fig. 2). We concluded that sequences required for cell cycle-specific timing were located between −143 and −60 bp and that elements required for high-magnitude expression were located between −205 and −143 bp of the *CYCB1;1* promoter.

Identification of Quantitative Elements by *in Vivo* Footprinting. Inspection of the DNA sequence between −143 and −60 bp revealed two copies of the previously identified MSA element required for G_2/M phase-specific timing of expression (17) (Fig. 1). We also identified a sequence weakly homologous to the CCAATCA-box and an auxin response factor (ARF)-binding site (Fig. 1). We used *in vivo* footprint analysis (25) to identify the binding sites of factors involved in *CYCB1;1* expression. We identified two classes of footprints. In *Arabidopsis* suspension-cultured cells or young leaves that still contained mitotic cells (6), we observed a hypersensitive site at GCC residues at −41 to −43 relative to the transcription start site (data not shown, Fig. 1), which was not observed in older, quiescent tissues.

A second class of footprint was identified, which defined an element with the consensus sequence GCCCR (R = G or A) (Fig. 7, which is published as supporting information on the PNAS web site). This element was protected from dimethyl sulfate modification in actively dividing cells, as well as in cells competent to divide, but not in quiescent cells in tissues such as mature leaves. This element is repeated four times in the *CYCB1;1* −351 promoter (Fig. 1). Three of the four copies of the

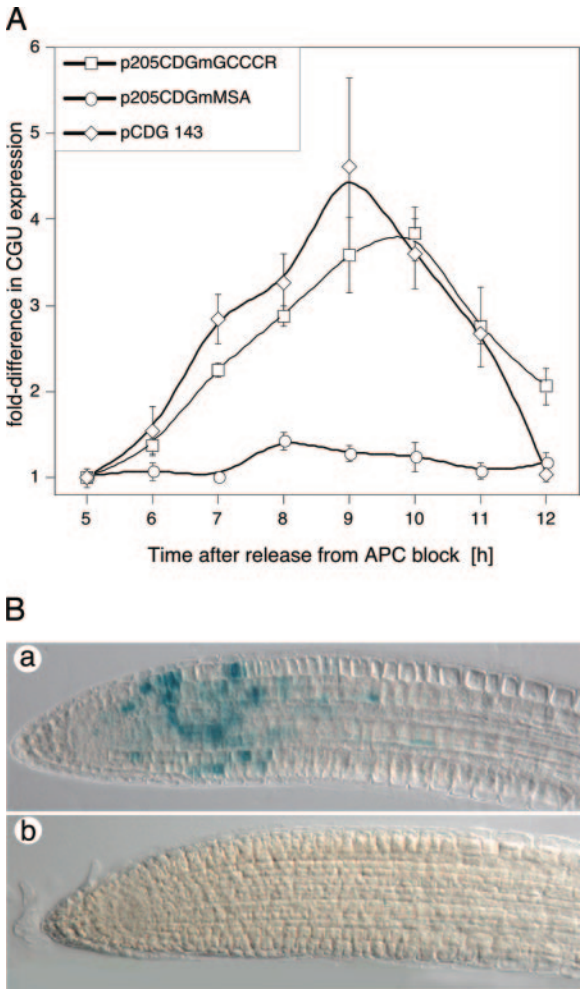

Fig. 3. Elements required for *CYCB1;1* expression in mitosis. The GCCCR elements within the −205 promoter were destroyed by site-directed mutagenesis and transformed into BY2 cells (*A*) or *Arabidopsis* plants (*B*). Mutation of the GCCCR element abrogates high-level *CYCB1;1* expression, but does not affect cell cycle phase-specific expression at G_2/M. However, mutation of the MSA elements within the −205 promoter quenches *CYCB1;1* expression at G_2/M. (*A*) Gene expression (left axis) relative to the values measured 5 h after release from the Aphidicolin block. (*B*) Histochemical analysis of GUS activity. (*a*) p205CDG-directed GUS activity. (*b*) p205CDGmGCCCR-directed GUS activity in roots. Identical results were obtained from 12 independently transformed lines.

GCCCR element are located between the −205 and −143 5′ deletion endpoints, raising the possibility that the cognate transcription factor is a major determinant of *CYCB1;1* transcript abundance.

Functional Analysis of Elements in Synchronized BY2 cells and *Arabidopsis* Plants. To test this possibility, the three GCCCR motifs between −143 and −205 were mutated to AAATR. The corresponding construct (pCDGmGCCCR) was introduced into BY2 cells and the accumulation of CGU RNA analyzed in synchronized cells (see Fig. 3*A*). CGU expression from the mutant promoter peaked at 9–10 h, coincident with the peak in mitotic activity (Fig. 2) and the maximum of p205CDG expression, but the magnitude of expression was low and similar to that observed in cells transformed with the p143CDG construct. In plants transformed with the AAATR mutant, we did not detect any GUS activity by histochemical analysis (Fig. 3*B*), and quantitative analysis by fluorescence spectrophotometry revealed a 20-fold reduction of GUS

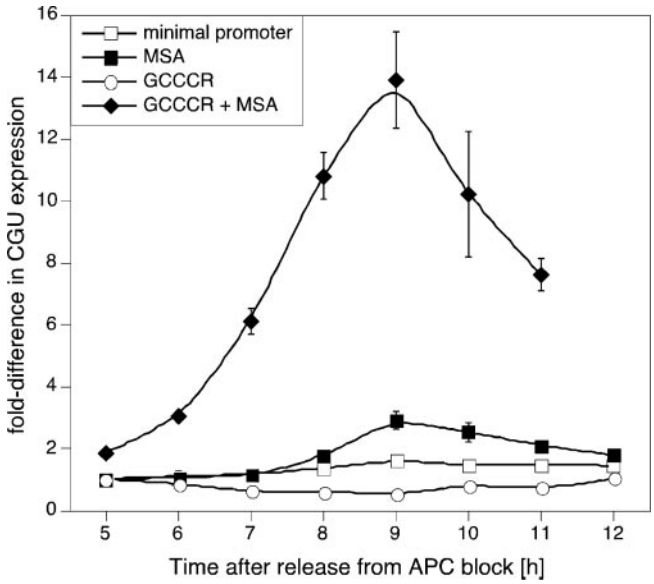

Fig. 4. The GCCCR element is necessary and sufficient to confer high-level *CYCB1;1* expression in mitosis. Oligonucleotides with the MSA element or the GCCCR element were cloned 5′ of the minimal 35S-46 promoter. The minimal promoter is not cell cycle phase-regulated, but addition of the MSA element restores expression at G_2/M. Addition of the GCCCR element alone slightly suppresses expression directed by the minimal promoter. However, addition of the GCCCR element to the MSA element strongly enhances expression with appropriate timing at G_2/M.

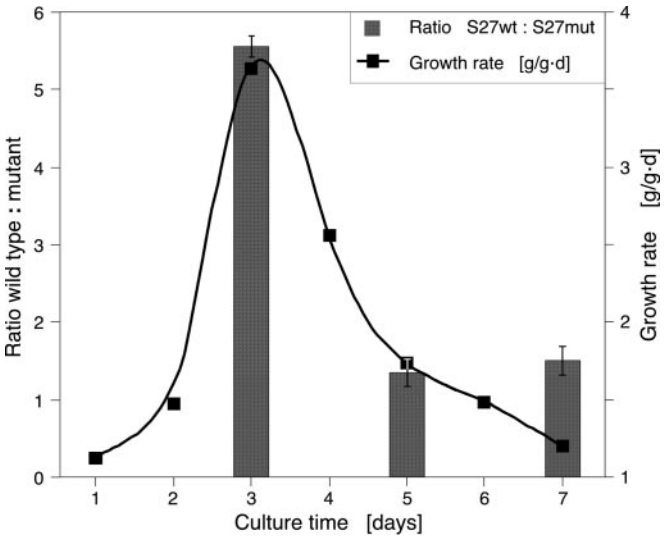

Fig. 5. The GCCCR element is necessary for high-level ribosomal protein gene expression. The promoter of RPS27aB was mutated to remove GCCCR motifs. Corresponding wild-type and mutant promoter–reporter constructs were introduced into BY2 cells. Growth rate was determined by measuring fresh weights of cultures. RPS27aB gene expression is more dependent on GCCCR motifs at high growth rates.

activity in root tips of these plants when compared to the wild-type (data not shown). We concluded that the GCCCR motif was necessary for high-level *CYCB1;1* expression at G_2/M. We also examined whether the MSA elements (Fig. 1) were required to confer G_2/M-specific expression in the context of the −205 promoter. Previous work had shown that this motif is required for G_2/M phase expression in *Catharanthus roseus* cyclin *CYM*, when assayed in BY2 cells (17, 34). The AACGG core MSA motif was mutated to ACTAG or AGCTG. CGU expression in BY2 lines transformed with this construct was not cell cycle regulated (Fig. 3*A*), indicating that the MSA motif is necessary for G_2/M-specific *CYCB1;1* expression.

To examine whether the GCCCR motif was sufficient to confer high-level induction of *CYCB1;1* expression, we generated a series of synthetic promoters on the basis of the −46 minimal 35S promoter. When expressed under control of the minimal promoter, CGU RNA did not exhibit any cell cycle regulation and addition of the GCCCR motif alone slightly suppressed the steady-state levels of CGU RNA (Fig. 4). However, when three copies of the MSA motif were added to the minimal promoter, G_2/M phase regulation of CGU abundance was observed, which was strikingly enhanced, when five copies of the GCCCR motif were added 5′ to the MSA elements (Fig. 4). Thus, the GCCCR motif is necessary and sufficient to enhance G_2/M expression mediated by MSA elements.

Genomic Analysis of the GCCCR Element in *Arabidopsis*. We next examined the distribution of GCCCR elements in promoters of *Arabidopsis* genes. Genes with the GCCCR element in their promoters were assigned to functional categories based on the scheme developed by the Munich Information Center for Protein Sequences (35). The frequency of each functional term in the list of genes was then compared to the frequency in the whole genome, and a *P* value of overrepresentation was calculated by using the hypergeometric distribution (R.A.G., L. Lejay, and G. M. Coruzzi, unpublished data). This analysis revealed that ribosomal protein, ribosome biogenesis, translation, and protein synthesis functions were significantly overrepresented (*P* value < 0.0001) in genes with three to four copies of the GCCCR element (Fig. 8, which is published as supporting information on the PNAS web site). Strikingly, genes with similar functions are induced when *Arabidopsis* plants are exposed to optimal concentrations of nitrogen and carbon, which strongly stimulate growth (R.A.G., L. Lejay, and G. M. Coruzzi, unpublished data). This finding raised the possibility that a subset of cell cycle genes, e.g., cyclins, and genes required for cell growth are coordinately regulated by shared transcription factors.

GCCCR Elements Are Required for High-Level Ribosomal Protein Expression. We then examined whether GCCCR elements in promoters of ribosomal protein (RP) genes were also required for their high-level expression. We selected two ribosomal protein genes, *RPS27aB* (At3g46040) and *RPL24B* (At3g53020), with six and five GCCCR motifs, respectively, and mutated these elements. Constructs expressing the CGU reporter under control of wild-type or mutant promoters were introduced into BY2 cells. High-level *RPS27aB* expression was strongly dependent on the GCCCR motif, specifically when growth rates were high during the culture cycle (Fig. 5). High-level expression of *RPL24B* was also dependent on GCCCR elements, but we observed less correlation with culture growth rates. We concluded that, as for *CYCB1;1*, high-level expression of these two RP genes depended on intact GCCCR elements.

p33^{TCP20} Binds to the GCCCR Element *in Vitro* and *in Vivo*. Class I teosinte-branched, cycloidea, PCNA factor (TCP) transcription factors bind to GGNCCCAC consensus sites (36), and the GCCCR element identified here forms a subset of these. Therefore, we examined whether TCP20, which had been shown to bind the *Arabidopsis PCNA2* promoter (37), interacts with the GCCCR elements in the *CYCB1;1* promoter in EMSAs. Recombinant TCP20 (p33^{TCP20}) binds specifically to the GCCCR elements in the *CYCB1;1* promoter (Fig. 9, which is published as supporting information on the PNAS web site), and is competed by an excess of unlabelled probe but cannot be competed with mutant probes.

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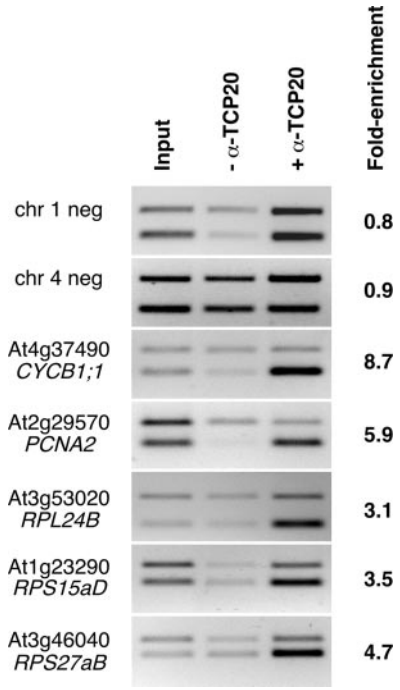

Fig. 6. $p33^{TCP20}$ coregulates cell cycle and ribosomal protein genes. Chromatin cross-linked to DNA isolated from *Arabidopsis* seedlings was immunoprecipitated with anti-$p33^{TCP20}$ antibodies. PCRs were performed with input DNA (*Left*), DNA precipitated without addition of anti-$p33^{TCP20}$ antibodies (*Center*), and DNA immunoprecipitated with anti-$p33^{TCP20}$ antibodies (*Right*). All reactions were performed in the presence of an internal control used to normalize reactions (top band), corresponding to sequences within At5g03545 (At4). Enrichment was calculated after normalizing input and immunoprecipitated reactions and comparing their ratios. Gene identifiers are shown at left.

We then used chromatin immunoprecipitation assays to examine whether $p33^{TCP20}$ binds *in vivo* to the *CYCB1;1* and promoters of other genes involved in executing growth programs with GCCCR motifs. Antibodies raised in sheep were used to precipitate $p33^{TCP20}$ cross-linked with DNA isolated from 12-day-old *Arabidopsis* seedlings. Subsequently, primers specific for *CYCB1;1*, *PCNA2*, *RPL24B*, *RPS15aD*, and *RPS27aB* were used to determine whether these genes are $p33^{TCP20}$ targets (Fig. 6). As controls, we examined $p33^{TCP20}$ binding to two genomic fragments on chromosome 1 and 4, which were >2 or >1.4 kb distant from any GCCCR motif, respectively. The negative controls showed no enrichment of templates recognized by the anti-$p33^{TCP20}$ antibody, whereas *CYCB1;1* and *PCNA2* were specifically enriched by 8.7- and 5.9-fold, respectively (Fig. 6). DNA fragments corresponding to the ribosomal proteins were also enriched (Fig. 6). We conclude that $p33^{TCP20}$ coregulates the expression of a suite of cell cycle control and ribosomal protein genes.

Discussion

We report the first mechanistic links between the regulation of cell growth and division in plants. The *Arabidopsis* TCP20 gene product, $p33^{TCP20}$, binds *in vivo* to cognate GCCCR elements in the promoters of *CYCB1;1,* of *PCNA2*, as well as the RPL24B, RPS15aD, and RPS27aB ribosomal protein genes we examined. GCCCR elements are required for high-level expression of the cyclin and ribosomal protein genes we examined.

Almost half (40%) of all *Arabidopsis* RP genes carry several clustered GCCCR motifs in their proximal promoters, suggesting that TCP-gene mediated transcriptional regulation of RP gene expression is likely to significantly contribute to the regulation of ribosome biogenesis. Most RP genes in *Arabidopsis* comprise small gene families. It is striking that, with two exceptions, GCCCR motifs are present in the promoters of only one member per RP gene family, suggesting that RP gene duplication has enabled their promoters to be differentially regulated. Moreover, the different temporal expression patterns of genes with GCCCR motifs (for example, *PCNA2* expression peaks in S phase, whereas the maximum of *CYCB1;1* expression is in G_2/M; ref. 32) suggests that the cognate TCP factors are likely to interact with different specific transcription factors to mediate high expression levels. However, we were not able to demonstrate *in vivo* $p33^{TCP20}$ binding to all promoter sequences with GCCCR motifs. For example, *Arabidopsis* cyclin *CYCA3;4* (At1g47230) and the transcription factor E2Fc (At1g47870) did not show enrichment in ChIP assays (data not shown). It is possible that $p33^{TCP20}$ does not bind to these promoters *in vivo*, that these factors are not coexpressed in the same cells, or alternatively, that the epitope recognized by the anti-$p33^{TCP20}$ antibody was occluded in the cross-linked tissue samples.

TCP20 is a class I TCP gene, and its closest paralogs in *Arabidopsis* are TCP6 and -11, which also bind to the GCCCR motif present in the *CYCB1;1* promoter *in vitro* (data not shown). These three genes, and possibly additional class I genes, may be functionally partially redundant, because the TCP20 knockout mutant has no obvious growth phenotype (C.L. and P.D., unpublished data). Class I TCP genes positively regulate gene expression (38), whereas class II TCP genes, such as *Arabidopsis* TCP2 and -4 or *Antirrhinum majus CYCLOIDEA*, *DICHOTOMA*, and *CINCINNATA* negatively regulate proliferation (8, 39–41). Precise spatio-temporal regulation of class II gene RNA accumulation is critical for leaf growth and morphogenetic development (8, 39). Interestingly, the DNA sequences recognized by class I (GGNCCCAC) and class II (GGNCCC) TCP genes are not mutually exclusive (36). Strikingly, we found that, in promoters with three or four GCCCR motifs (Fig. 8), these are much more likely to be nested within a GGNCCC motif than expected by chance alone ($P < 0.0001$). This finding raises the attractive possibility that both classes of TCP genes share target genes.

We propose a model in which class I TCP factors mediate the marked stimulation of cell growth and division required for elevated cell production rates in young lateral primordia in shoots (6) or in the multiplicative division zone of the root meristem (7). This elevation would be followed by concerted suppression of cell growth and division by class II TCP genes as cells exit the multiplicative zone. Thus, organ growth is proposed to be regulated by the balance of antagonistic activities of class I and II TCP genes. In this model, the crucial variable underpinning organ growth is the population size of dividing cells within the zone of multiplicative divisions. Indeed, kinematic analysis of root growth reveals that, although there is no evidence for shortening cell cycle duration when root growth accelerates, there is clear evidence that the multiplicative division zone expands distally as root organ growth accelerates (9, 42). This finding highlights the importance of controlling the exit from this zone for overall organ growth rate in roots and organ shape and morphology in shoots. It will be interesting to test this model by examining the balance of TCP gene activities in this boundary zone.

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