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A Genetic Screen for Dominant Modifiers of a *cyclin E* Hypomorphic Mutation Identifies Novel Regulators of S-Phase Entry in Drosophila

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ABSTRACT

Cyclin E together with its kinase partner Cdk2 is a critical regulator of entry into S phase. To identify novel genes that regulate the G1- to S-phase transition within a whole animal we made use of a hypomorphic *cyclin E* mutation, *DmcycE^{JP}*, which results in a rough eye phenotype. We screened the *X* and third chromosome deficiencies, tested candidate genes, and carried out a genetic screen of 55,000 EMS or X-ray-mutagenized flies for second or third chromosome mutations that dominantly modified the *DmcycE^{JP}* rough eye phenotype. We have focused on the *DmcycE^{JP}* suppressors, *S*(*DmcycE^{JP}*), to identify novel negative regulators of S-phase entry. There are 18 suppressor gene groups with more than one allele and several genes that are represented by only a single allele. All *S*(*DmcycE^{JP}*) tested suppress the *DmcycE^{JP}* rough eye phenotype by increasing the number of S phases in the postmorphogenetic furrow S-phase band. By testing candidates we have identified several modifier genes from the mutagenic screen as well as from the deficiency screen. *DmcycE^{JP}* suppressor genes fall into the classes of: (1) chromatin remodeling or transcription factors; (2) signaling pathways; and (3) cytoskeletal, (4) cell adhesion, and (5) cytoarchitectural tumor suppressors. The cytoarchitectural tumor suppressors include *scribble*, *lethal-2-giant-larvae* (*lgl*), and *discs-large* (*dlg*), loss of function of which leads to neoplastic tumors and disruption of apical-basal cell polarity. We further explored the genetic interactions of *scribble* with *S*(*DmcycE^{JP}*) genes and show that hypomorphic *scribble* mutants exhibit genetic interactions with *lgl*, *scab* (αPS3-integrin—cell adhesion), *phyllopod* (signaling), *dEB1* (microtubule-binding protein—cytoskeletal), and *moira* (chromatin remodeling). These interactions of the cytoarchitectural suppressor gene, *scribble*, with cell adhesion, signaling, cytoskeletal, and chromatin remodeling genes, suggest that these genes may act in a common pathway to negatively regulate *cyclin E* or S-phase entry.

REGULATION of the G1- to S-phase transition by external signals is critical to the decision to proliferate or to differentiate. Progression through G1 phase is controlled by the activity of the Cyclin-dependent ser/thr protein kinases (Cdks) associated with their regulatory Cyclin partners (Ekholm and Reed 2000). In mammalian cells, the G1 cyclins, Cyclin D (D1, D2, and D3) in association with Cdk4(6), and Cyclin E (E1 and E2) in association with Cdk2, play distinct roles in the G1- to S-phase transition. Cyclin D/Cdk4 functions early in G1, while cyclin E/Cdk2 functions at the G1- to S-phase transition, triggering DNA replication initiation and centrosome duplication. In mammalian cells, Cyclin D/Cdk4 and Cyclin E/Cdk2 act to phosphorylate and inactivate the tumor suppressor protein, Retinoblastoma (Rb; Lundberg and Weinberg 1998), which functions by binding to and inactivating the E2F/Dp transcription factor required for the transcription of S-phase genes (Dyson 1998). Binding and phosphorylation of Rb by Cyclin D/Cdk4 and Cyclin E/Cdk2 inactivate Rb, allowing the E2F/Dp transcription factor to function. G1 Cyclin-Cdks are also regulated by the binding of Cdk inhibitory proteins (Sherr and Roberts 1999), such as the p21^{CIP1} class of inhibitors, which bind to Cyclin E/Cdk2, inhibiting its activity and leading to G1 arrest.

The key players in the regulation of the G1- to S-phase transition are highly conserved between mammals and Drosophila (Edgar and Lehner 1996). Genetic analysis in Drosophila has shown that both Cyclin E and Cyclin D act to regulate Drosophila Rb (Rbf; Xin *et al.* 2002). Drosophila Cyclin E is essential for the G1- to S-phase transition during embryogenesis and is downregulated in G1-arrested cells (Richardson *et al.* 1993, 1995; Knoblich *et al.* 1994). In contrast, Drosophila Cyclin

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D primarily acts to regulate cell growth (increase in cell mass) and through the coupling of cell growth to G1- to S-phase progression, stimulates cell proliferation (Datar *et al.* 2000; Meyer *et al.* 2000). As in mammalian cells, Drosophila Cyclin E/Cdk2 activity is regulated via a homolog of $p21^{CIP1}$, Dacapo, which is required during exit into a terminal G1 arrest prior to differentiation (de Nooij *et al.* 1996; Lane *et al.* 1996). Degradation of Cyclin E protein also plays an important role in limiting cell proliferation, and mutations in the *ago* gene (encoding a homolog of Cdc4, an F-box-containing component of the G1 phase ubiquitin ligase) result in increased Cyclin E protein stability and excessive cell proliferation during eye development (Moberg *et al.* 2001). However, relatively little is known about the upstream signals that regulate Drosophila *cyclin E* transcription or the downstream targets of Drosophila Cyclin E/Cdk2 that lead to the initiation of DNA replication within a whole-animal context.

The developing Drosophila eye presents an ideal system to study the relationship between cell proliferation and differentiation. The eye develops from a single cell layer epithelium at the third larval instar stage, where a wave of morphogenesis moves from the posterior to the anterior of the eye imaginal disc (Thomas and Wassarman 1999). Associated with this wave of morphogenesis is the morphogenetic furrow (MF), where the cell cycle becomes coordinated with differentiation. Within and anterior to the MF cells are arrested in G1, while posterior to the MF a subset of cells begins to differentiate into the photoreceptor cell preclusters and the surrounding cells enter a synchronous S phase, after which a subset of these cells undergoes mitosis. Hedgehog signaling has been shown to be important for Cyclin D and Cyclin E expression in this post-MF cell division (Duman-Scheel *et al.* 2002). Perturbations to the organized arrangement of cell division in the developing eye by, for example, ectopic expression of S-phase inducers, Cyclin E or E2F/Dp, or the negative cell cycle regulators, human p21 or Drosophila Rbf, result in defects in eye development leading to disorganized or rough adult eyes (de Nooij and Hariharan 1995; Richardson *et al.* 1995; Asano *et al.* 1996; Du *et al.* 1996; Xin *et al.* 2002). The eye phenotypes resulting from overexpression of Cyclin E, E2F/Dp, or Rbf in the posterior differentiating cells of the eye disc have been used as the basis of genetic screens of EMS-mutagenized flies to identify dominant modifiers, revealing novel regulators of the cell cycle (Staehling-Hampton *et al.* 1999; Boulton *et al.* 2000; Lane *et al.* 2000; Duman-Scheel *et al.* 2002).

A hypomorphic mutation in Drosophila *cyclin E, DmcycE^{JP}*, which results in a rough eye phenotype, has provided an opportunity to carry out genetic screens to identify novel genes involved in the regulation of *cyclin E* expression and function. We have previously shown that *DmcycE^{JP}* exhibits a rough eye phenotype due to a reduction in Cyclin E levels and S phases in the developing eye and that this phenotype is sensitive to the dosage of G1- to S-phase genes known to interact with Cyclin E (Secombe *et al.* 1998). This article reports the results of mutagenesis and deficiency screens to identify genes that dominantly modify the *DmcycE^{JP}* rough eye phenotype and presents initial characterization of *DmcycE* suppressor genes, predicted to act as negative regulators of Cyclin E and/or the G1- to S-phase transition.

MATERIALS AND METHODS

Mutagenesis screen: For X-ray mutagenesis, 3- to 5-day-old Drosophila males were placed into empty vials (~100 in each) and treated with 4000 rad of X rays in a CIS Biointernational X-ray machine using a ^{137}Cs radiation source (activity 3400 Ci). Mutagenized flies were then allowed to recover for 4 hr with food before being added to 3-day-old virgin females. The flies were turned into new bottles after 2 days and removed after 4 days. EMS mutagenesis was carried out as previously described (Grigliatti 1998). For both EMS and X-ray mutageneses, *DmcycE^{JP}* males isogenic on the second and third chromosomes were mutagenized and crossed *en masse* to *b DmcycE^{JP}* females. The progeny from this cross were scored for dominant modification of the *DmcycE^{JP}* rough eye phenotype. In addition, F_1 progeny were scored for black-bodied flies to estimate the mutation frequency. From the number of black mutant flies obtained, we calculated that the X-ray mutagenesis frequency was 2.3×10^{-3} and the EMS mutagenesis frequency was $\sim 3 \times 10^{-4}$, which are within the ranges described by previous studies (Grigliatti 1998).

Flies selected as having enhanced or suppressed eyes were crossed to a *DmcycE^{JP}* strain to ensure that the modification of the *DmcycE^{JP}* rough eye phenotype observed initially was heritable and reproducible and then crossed to second or third chromosome balancers to generate stocks. To simplify the screen and stock generation, only interactors that mapped to the second or third chromosome were kept. Once a stock was generated, flies were crossed to a *DmcycE^{JP}* strain to ensure that the enhancer or suppressor mutation segregated away from the balancer chromosome. Any mutations that resulted in a dominant eye roughening in the absence of *DmcycE^{JP}* were discarded.

For complementation analysis, *inter se* crosses were carried out between all lethal alleles on each chromosome and allele combinations that resulted in *trans*-heterozygous lethality to >98% were considered to be within the same gene group.

Genetic mapping of second chromosome genes was carried out using either the b^1, cn^1, bw^1 or al^1, dp^{ov1}, b^1, pr^1, c^1, px^1, sp^1 multiply marked chromosomes, while third chromosome genes were mapped using the ru^1, h^1, th^1, st^1, cu^1, sr^1, e^s, ca^1 multiply marked chromosomes. The deficiency kit (Bloomington Stock Center) was used for deficiency mapping. Chromosome cytology of third chromosome suppressors was analyzed after Giemsa staining of polytene chromosomes prepared from non-Tubby larvae from a cross of the suppressor (over *TM6B*) to Canton-S.

***DmcycE* interactions:** To test *X* and third chromosome deficiencies for interaction with *DmcycE^{JP}*, stocks were generated using balancers that contained the deficiency chromosome and *DmcycE^{JP}* and the stock was crossed to homozygous *DmcycE^{JP}* flies and progeny containing the deficiency and *DmcycE^{JP}* were examined. To examine second chromosome candidate genes for interaction with *DmcycE^{JP}*, the candidate gene mutant was recombined onto a marked *DmcycE^{JP}* chromosome (using a *dp, b, DmcycE^{JP}, cn, bw* chromosome) balanced over *CyO* and then crossed to a homozygous *DmcycE^{JP}* stock and non-Curly

flies were examined. *X* or third chromosome candidate genes were tested for interaction with $DmcycE^{JP}$ after generating stocks containing the candidate gene mutant and $DmcycE^{JP}$, by crossing to *b*, $DmcycE^{JP}$, *bw* flies and examining non-*FM7* or non-*TM6B* flies. The eyes of at least 50 flies of the appropriate genotype were examined and compared with *b*, $DmcycE^{JP}$, *bw*/$DmcycE^{JP}$ flies.

Phenotypic analysis of *cyclin E* suppressors: To determine whether a suppressor was acting at the level of S-phase regulation, second chromosome modifiers were crossed to the *Curly-Tubby* (*Cy-Tb*) second chromosome balancer, which carries the *Tubby* dominant larval marker, and were crossed to homozygous $DmcycE^{JP}$ flies, and non-Tubby larvae were selected for examination of S phases by BrdU labeling. Third chromosome modifiers were balanced over *TM6B* (marked by *Tubby*) and crossed to homozygous $DmcycE^{JP}$ flies and non-Tubby larvae were picked for BrdU labeling. BrdU labeling was carried out as described previously (Secombe *et al.* 1998). Cyclin E antibody staining was carried out using a polyclonal Cyclin E antibody raised in rats, as previously described (Crack *et al.* 2002). To determine whether the stage of lethality was before or after the third instar larval stage, each suppressor stock balanced over *Cy-Tb* or *TM6B* was examined for the presence of any homozygous modifier (non-Tubby) larvae that survived to or beyond the third larval instar stage. Scanning electron microscopy of adult eyes was carried out as previously described (Secombe *et al.* 1998).

RESULTS

Identification of *X* and third chromosome deficiencies that dominantly modify $DmcycE^{JP}$: We have previously demonstrated that the $DmcycE^{JP}$ rough eye phenotype is sensitive to the gene dose of known *cyclin E*-interacting genes (Secombe *et al.* 1998). To obtain an estimate of how many interactors were expected from a random mutagenesis, available *X* and third chromosome deficiencies were tested to determine how many of these were able to modify the $DmcycE^{JP}$ phenotype.

A total of 20 suppressor regions and 16 enhancer regions on the *X* and third chromosomes were identified by generating homozygous $DmcycE^{JP}$ flies that were also heterozygous for the deficiency chromosome (Table 1). Consistent with results described previously (Secombe *et al.* 1998), deficiencies removing genes already known to interact with $DmcycE^{JP}$ such as *RBF*, *roughex*, *E2F1*, and *string* behaved as expected (Table 1), with the exception of *Df(3R)vin2* that removes *cyclin A*. We have previously shown that *cyclin A* mutants dominantly enhance $DmcycE^{JP}$ phenotype (Secombe *et al.* 1998), while *Df(3L)vin2* and the overlapping deficiency *Df(3L)vin5* suppressed the $DmcycE^{JP}$ rough eye phenotype. The most likely explanation for this is that these deficiencies also delete a dose-sensitive suppressor of $DmcycE^{JP}$ (Table 1). Suppression of $DmcycE^{JP}$ was also observed with a deficiency (of the region 63F4–64C15) removing the Drosophila *cdc4(ago)* gene, which encodes an F-box protein of the Skp1-cullin-F-box (SCF) ubiquitin ligase complex involved in Cyclin E protein degradation and can dominantly suppress the $DmcycE^{JP}$ rough eye phenotype (Moberg *et al.* 2001).

For the remaining $DmcycE^{JP}$ interacting deficiencies, the regions were searched for possible candidate modifying genes using the cytosearch function at Flybase. Candidate *cyclin E*-interactors were genes expected to either promote S-phase entry for enhancers or inhibit S-phase entry for suppressors. These include homologs of tumor suppressors or oncogenes, genes involved in the initiation of DNA replication, in ubiquitin-mediated degradation pathways, or in chromatin remodeling (Table 1). Genes involved in chromatin remodeling were considered candidates, on the basis of the observation in mammalian cells that components of the SWI/SNF-Brahma chromatin remodeling complex negatively regulate cell proliferation (Harbour and Dean 2001). In a number of cases, specific mutations in these candidate genes were tested for modification of the $DmcycE^{JP}$ phenotype. This approach enabled the identification of a number of novel *cyclin E*-interacting genes (Table 1, and see below). For the most part, however, identification of candidates within the modifying deficiency, based on the expected classes of interactors, was not successful. Of the 36 regions that modify $DmcycE^{JP}$, candidate genes for only 12 of these were shown to modify $DmcycE^{JP}$ in a way that would account for the modification by the deficiency. In addition to *Rbf1*, *roughex*, *ago* (*cdc4*), *E2F1*, and *string* discussed above, *discs-large* (*dlg*), *RpS6*, *brahma*, *sina*, *Abl*, *scribble*, and *crumbs* were identified in this way and are discussed in detail below. Many interactors did not have an obvious candidate gene within the deficiency breakpoints, or possible candidates were tested but did not interact with *cyclin E*, or specific mutations were not available in the candidate genes.

Tumor suppressors and oncogenes: From the $DmcycE^{JP}$ deficiency screen, a number of regions that showed suppression removed Drosophila tumor suppressor genes, while many that enhanced removed potential oncogenes (Table 1). These candidate genes, as well as other potential oncogenes or tumor suppressors, were specifically tested where possible (Table 2).

Deficiencies removing potential oncogenes that enhanced $DmcycE^{JP}$ include those removing a Ras-like GTPase *Rac1* (61C3–4; 62A8), a Rap-related GTPase *Roughened/Rap1/dRas3* (62B8–9; 62F2–5), and a Ras-like GTPase *Ras64B* (63E1–2; 64B17). Loss-of-function mutations in *Rap1* did not affect $DmcycE^{JP}$ (not shown) and we have not yet tested *Rac1*. However, we have shown that *trio*, which encodes a Rac activator, dominantly suppresses $DmcycE^{JP}$ (see below). Moveover, *Rac2*, which plays a redundant role with *Rac1*, was isolated in a screen for genes that when overexpressed inhibit cell proliferation in the Drosophila eye, which was rescuable by ectopic expression of Cyclin E (Tseng and Hariharan 2002). Taken together these data suggest that Rac is a negative regulator of G1-S progression in Drosophila and thus it is unlikely that halving the dosage of Rac1 accounts for the dominant enhancement of the 61C3–4 to 62A8 region. We were also unable to test *Ras64B*

TABLE 1

***X* and third chromosome regions that modify the *DmcycE^{JP}* phenotype**

Deficiency	Region removed by deficiency	Effect on *DmcycE^{JP}*	Candidate genes in the region
		X chromosome	
Suppressors			
Df(1)tBA1	1A1 to 2A	Suppression	*Rbf*[a]
Df(1)JC19	2F6 to 3C5	Suppression	NC
Df(1)N73 and *Df(1)sqh*	5D1–2 to 5D5–6	Suppression	*roughex*, *air4* (−)
Df(1)KA14	7F1–2 to 8C6	Mild suppression	*lawc* (−), *air11*
Df(1)v^{N48}	9F to 10C3–5	Suppression	*dlg*
Df(1)C246	11D–E to 12A1–2	Suppression	*BAP60*[b]
Df(1)RK4	12F5–6 to 13A9–B10	Suppression	NC
Df(1)sc72b	13F1 to 14B1	Suppression	NC
Df(1)ma13	19A1–2 to 20E–F	Suppression	*RpS6*
Enhancers			
Df(1)4b18	14B8 to 14C1	Enhancement	NC
Df(1)N19	17A1 to 18A2	Enhancement	*fused*
		Third chromosome	
Suppressors			
Df(3L)HR232 and *Df(3L)HR119*	63C6 to 63D3	Suppression	*sprouty*
Df(3L)GN24	63F4–7 to 64C13–15	Suppression	*cdc4* (*ago*)[c]
Df(3L)vin2 and *Df(3L)vin5*	68A2–3 to 68D6	Suppression	NC *3.2* region (See Table 5)
Df(3L)brm11	71F1–4 to 72D1–10	Suppression	*Brahma 3.5* region (see Table 5)
Df(3L)81K19	73A3 to 74F	Suppression	*argos*, *Abl*, *Dab*, *sina*, *sinah*[d] *3.1* region (Table 5)
Df(3R)p712	84D4–6 to 85B6	Suppression	*pyd(Z01)*
Df(3R)by10	85D8–12 to 85F1	Suppression	*hyd* (−)
Df(3R)Cha7	90F1–4 to 91F5	Suppression	NC
Df(3R)XS	96A1–7 to 96A21–25	Mild suppression	NC
Df(3R)T1^{P}	97A to 98A1–2	Mild suppression	*l(3)mbt* (−), *scribble 63S15* region (Table 5)
Df(3R)awd^{KRB}	100C6–7 to 100D3–4	Suppression	*tramtrack* (−)
Enhancers			
Df(3L)emc5	61C3–4 to 62A8	Enhancement	*emc* (−), *trio* (S) *Rac1*[b]
Df(3L)R^{G7}	62B8–9 to 62F2–5	Enhancement	*Roughened* (*Rap1*) (−)
Df(3L)HR370	63A1 to 63D10	Enhancement	NC
Df(3L)GN50	63E1–2 to 64B17	Enhancement	*cdc2-63E* (−), *Ras64B*[b], *RfC40*
Df(3L)66C^{G28}	66B8–9 to 66C10	Enhancement	*DNApolα50*[b]
Df(3L)h^{i22}	66D10–11 to 66E1–2	Enhancement	*h* (−), *dally* (−), *mcm7*[b]
Df(3L)AC1	67A2 to 67D7–13	Enhancement	*shc*, *eif-4E*, *cdk8*[b]
Df(3L)Cat	75B8 to 75F1	Enhancement	Replication-deficient region[b]
Df(3L)rdgC	77A1 to 77D1	Enhancement	*DNA primase*
Df(3L)Pc^{MK}	78A3 to 79E1	Enhancement	*cyclin H DNApol-eta*[b]
Df(3R)Tp110	83C1–2 to 84B1–2	Enhancement	*plx*
Df(3R)e^{N19}	93B to 94A	Enhancement	*E2F1*
Df(3R)crbS87^{4}	95E8–F1 to 95F15	Enhancement	*crb*
Df(3R)3450	98E3 to 99A6–8	Enhancement	*string*

Where deficiencies that overlap have the same effect on *DmcycE^{JP}*, the region common to both deficiencies is given as the cytological region. Candidate genes that are underlined have the same effect on the *DmcycE^{JP}* phenotype as the corresponding deficiency. Those indicated by (−) have been tested and shown to have no effect on *DmcycE^{JP}*, while those indicated by (S) have been shown to suppress rather than enhance. NC, there was no candidate satisfying our criteria in the interval. Gene descriptions are as follows: *Rbf*, *Retinoblastoma*; *roughex* (inhibitor of Cyclin A/Cdk1 in the MF); *air4*, *aberrant immune response 4* (blood cell tumor suppressor); *lawc* (enhancer of *TxG* mutants); *air11*, *aberrant immune response 11* (blood cell tumor suppressor); *dlg*, *discs-large* (cytoarchetectual protein, neoplastic tumor suppressor); *BAP60*, *Brahma-associated protein 60* (Brahma complex protein, chromatin remodeling); *RpS6*, *Ribosomal protein S6* (translation factor, tumor suppressor); *fused* (protein kinase required for Hh signaling); *sprouty* (acts antagonistically to the Egfr); *brahma* (SWI2-related ATPase, chromatin remodeling, negative growth regulator); *argos* (Egfr ligand, anatogonist of Egfr signaling); *Abl* (nonreceptor tyrosine protein kinase); *dab*, *disabled* (acts synergistically with Abl); *sina* and *sinah* (ring finger E3 ubiquitin ligases, protein degradation); *pyd*, *ZO1*, and *tamou* (membrane-associated guanylate kinase); *hyd*, *hyperplastic discs* (HECT domain E3 ubiquitin ligase, protein degradation); *l(3)mbt*, *lethal* (*3*) *malignant brain tumor* (translation factor, tumor suppressor); *scribble* (cytoarchitectual protein, neoplastic tumor suppressor);

since there were no available mutants in this gene. However, we tested whether mutants in other oncogenic GTPases, Ras85D and Rho1, could enhance *DmcycE^{JP}* (Table 2). Ras has a well-established role in oncogenesis in mammalian cells (Malumbres and Barbacid 2003) and overexpression of an activated form of Ras85D in Drosophila results in a hyperplastic phenotype (Karim and Rubin 1998). Ras85D has also been shown to increase Cyclin E protein levels post-transcriptionally in the wing and eye discs (Prober and Edgar 2000; Brumby and Richardson 2003). Consistent with its expected role as positive regulator of G1-S progression, mutations in *Ras85D* dominantly enhanced the *DmcycE^{JP}* rough eye phenotype (Table 2; data not shown). In mammalian cells, Rho promotes cell proliferation and is required for Ras-induced transformation (Sahai and Marshall 2002). Indeed, overexpression of wild-type and dominant active forms of mammalian Rho have been shown to upregulate Cyclin E/Cdk2 activity and induce progression from G1 into S phase. Although, no role for Rho1 has been revealed in G1-S progression in Drosophila, we observed that mutants in *rho1* dominantly enhanced the *DmcycE^{JP}* rough eye phenotype (Table 2; data not shown), revealing a novel role for Drosophila Rho1 that warrants further investigation.

In addition, a deficiency removing *fused* (17A1–18A2), an effector of the Hh pathway, showed enhancement of *DmcycE^{JP}*. Although we have not specifically tested *fused* to determine whether it represents the interacting gene, this interaction is consistent with the recent observation that the Hedgehog (Hh) pathway acts to upregulate *cyclin E* transcription in the eye (Duman-Scheel *et al.* 2002) and that upregulation of the Hh pathway is oncogenic in mammals (Wetmore 2003). To explore this further, we analyzed the effect of halving the dose of *Hh* or *patched* (a negative regulator of the Hh receptor, Smoothened) on the *DmcycE^{JP}* rough eye phenotype (Table 2; data not shown). As expected, Hh alleles dominantly enhanced while *patched* alleles dominantly suppressed *DmcycE^{JP}*, consistent with a role for the Hh pathway in positively regulating *cyclin E* and inducing S-phase entry. We also examined other signaling pathways for dominant interactions with *DmcycE^{JP}* (Table 2; data not shown). In mammalian cells, the EGF receptor, the Wnt/Wingless, and Notch signaling pathways have a growth and/or cell cycle stimulatory role in many cells and can be oncogenic when upregulated (Allenspach *et al.* 2002; Chang *et al.* 2003; Giles *et al.* 2003). Consistent with the interaction of *Ras85D* with *DmcycE^{JP}*, loss-of-function mutations in the EGF receptor (Egfr) enhanced *DmcycE^{JP}* while gain-of-function mutations (Ellipse) suppressed. Other downstream components of the Egfr-Ras pathway also interacted with *DmcycE^{JP}* in a manner consistent with the Egfr having a positive role in regulating Cyclin E and entry into S phase (Table 2). Reducing the dose of *Notch*, however, showed no effect on the *DmcycE^{JP}* phenotype. Interestingly, halving the dosage of *wingless* (*wg*), *disheveled* (encoding a Wg-signaling mediator), and *armadillo* (*arm*; encoding a β-catenin homolog, the Wg signaling transcriptional effector) resulted in suppression of *DmcycE^{JP}*. In contrast, halving the dosage of *axin* (encoding an inhibitor of Wg signaling) enhanced *DmcycE^{JP}*. While contrary to the expected role of the Wg pathway, an inhibitory proliferative function for Wg has been observed in the zone of nonproliferation in the third instar wing pouch (Johnston and Edgar 1998; Johnston *et al.* 1999; Johnston and Sanders 2003). Similarly, we have previously shown that the Dpp (TGFβ homolog), although growth stimulatory earlier in development, acts to negatively regulate cell cycle progression in the third instar eye imaginal disc and mutants that disable the Dpp signaling pathway dominantly suppress *DmcycE^{JP}* (Horsfield *et al.* 1998).

Several *DmcycE^{JP}* suppressor regions on the *X* chromosome and on the third chromosome remove known

TABLE 1

(Continued)

tramtrack (neural differentiation inhibitor); *emc*, *extra-macrochaetae* (Id-related HLH repressor protein required for cell proliferation in the wing and with *hairy* for MF progression in the eye); *trio* (Rac-GEF, required for Rac activation); *Rac1* (Rac family GTPase); *Roughened* (Rap1; Ras-like GTPase); *cdc2-63E* (cdc2-related protein kinase); *Ras64B* (Ras-related); *RfC40, Replication factor-C40* (DNA replication initiation); *DNApolα50, DNA polymerase-α 50-kD subunit* (DNA replication); *h*, *hairy* (see *emc*); *dally* (glypican, cooperates with Wg and other growth factor receptors); *mcm7, minichromosome maintenance 7* (DNA replication initiation); *shc* (adaptor protein required for Egfr signaling); *eif-4E* (translational initiation factor); *cdk8* (cdc2-related protein kinase); DNA primase (DNA replication); *cyclin H* (Cyclin required for activation of cdk8 protein kinase); *DNApol-eta* (DNA replication); *plx*, pollux (a cell adhesion protein related to the human oncogene TRE17; Zhang *et al.* 1996); *E2F1* (S-phase transcription factor); *crb, crumbs* (apical-lateral membrane protein involved in cell polarity); and *string* (Cdc25 phosphatase, activator of Cdc2). An unidentified gene essential for DNA replication is located within the 75B8–75F1 region (Smith *et al.* 1993). For more details see text.

[a] Specific alleles of *RBF* have been shown to suppress the *DmcycE^{JP}* phenotype and overexpression of *RBF* enhances the *DmcycE^{JP}* rough eye phenotype (Secombe *et al.* 1998; our unpublished data).

[b] No specific mutation is available to test the interaction.

[c] *cdc4* (*ago*) alleles have been shown to suppress *DmcycE^{JP}* (Moberg *et al.* 2001).

[d] *sina* alleles showed only weak dominant suppression of the *DmcycE^{JP}* rough eye phenotype; however, a *sina*-related gene is located next to *sina* (*sinah*), and removal of both may account for the suppression observed by the deficiency (M. Coombe, L. Quinn, R. Dickins, J. Secombe and H. Richardson, unpublished results).

TABLE 2

Interaction of tumor suppressor and signaling pathway mutants with *DmcycE^{JP}*

Gene: allele	Function	Effect on *DmcycE^{JP}*
Tumor suppressors		
dlg: *dlg^{6}*	Cell polarity	Suppression
l(3)mbt: *l(3)mbt^{E2}*		No effect
brat: *l(2)37Cf^{1}*	Translation	No effect
air2		No effect
air4		No effect
air6		Mild suppression
air7		Suppression
air8 (RpS6)	Translation	Suppression
air10		Suppression
air13		No effect
hop-air (activated allele of Jak)	Mitogenic signaling	Suppression
air16		Mild suppression
lats/warts: *lats^{P1,91}*	Protein kinase	No effect
hyd: *hyd^{15}*	HECT domain Ubiquitin ligase	No effect
fat: *fat$^{4,G\text{-}rv}$*	Atypical Cadherin	Suppression
expanded: *ex^{01270}*	FERM domain 4.1 superfamily	Suppression
shotgun: *shg^{k03401,2}*	E-cadherin	Mild suppression
Negative regulators of signaling pathways		
patched: *ptc^{IIIa,G12}*	Inhibitor of Hh signaling	Suppression
Gap1: *Gap1^{1}*	Ras-GAP—inactivates Ras	Suppressed
yan/aop: *aop^{1}*	Inhibitor of Ras pathway—Pnt transcription upregulation	No effect
axin: *axn^{E77}*	Inhibitor of Wg signaling	Mild enhancement
Positive regulators of signaling pathways		
Egfr-top^{QV1}—hypomorphic	Receptor tyrosine protein kinase	LOF—enhancement
Elp^{B1}—ligand independent activated allele	(RTK)	GOF—suppression
Ras85D: *Ras85^{e1b}*	Effector of Egfr signaling	Enhancement
drk: *drk^{10626}*	Effector of Egfr signaling	Slight enhancement
pointed: *pnt^{7825}*	Transcription factor—downstream of Ras signaling	Slight suppression
rhoA: *rhoA^{72R,720}*	Actin cytoskeleton reorganization	Enhancement
Roughened (*Rap1*): *Rap1^{CD3}*	Actin cytoskeleton reorganization	LOF—no effect
Notch: *N$^{264\text{-}39}$*	Signaling protein	No effect
hedgehog: *hh^{18,21,AC}*	Signaling protein	Mild enhancement
wingless: *wg$^{IL1\text{-}8,1\text{-}17}$*	Signaling protein	Mild suppression
armadillo: *arm^{YD35}*	Transcriptional factor mediator of Wg signaling	Suppression
disheveled: *dsh^{3,6}*	Mediator of Wg signaling	Mild suppression
spen (*poc*): *poc$^{261\text{-}18,361\text{-}6}$*	RNA-binding protein	Suppression

LOF, loss of function; GOF, gain of function.

Drosophila tumor suppressor genes. Specific mutations were available for some of the candidate genes encoding tumor suppressors and were therefore tested for a genetic interaction with *cyclin E*. Specific mutations in *Ribosomal protein S6* (*RpS6 air8*), the best candidate for the *cyclin E* suppressor in the 19A–20F region, were tested and shown to suppress the *DmcycE^{JP}* rough eye phenotype (Table 2; not shown). Mutations in *RpS6* were identified as loss-of-function mutations that result in overproliferation of larval hematopoietic tissues and give rise to variable melanotic tumor phenotypes (Gateff *et al.* 1996). RpS6 is phosphorylated in response to mitogen stimulation and phosphorylated RpS6 is preferentially incorporated into polysomes, resulting in an increased rate of translation of a subset of transcripts (Amaldi and Pierandrei-Amaldi 1997; Martin and Blenis 2002). However, disruption of Drosophila S6 kinase leads to reduced growth and smaller flies and mutation of the upstream kinase Tor causes cell cycle arrest that can be rescued by *cyclin E* expression (Zhang *et al.* 2000). Furthermore, conditional knockout of RpS6 in mice results in a specific block in *cyclin E* expression (Volarevic *et al.* 2000). Given this role for RpS6 in mammalian cells, it is unknown how halving the dosage of RpS6 leads to the suppression of *DmcycE^{JP}*; however, it is consistent with the tumor suppressor function of Drosophila RpS6.

Other Drosophila tumor suppressors were tested for interaction with *DmcycE^{JP}* (Table 2), and those that showed suppression were *hop-air* (an activating mutation

TABLE 3

Summary of the 246 modifiers identified in the screen

	Second chromosome		Third chromosome	
	Homozygous lethal	Homozygous viable	Homozygous lethal	Homozygous viable
Suppressors				
EMS	19	5	5	0
X ray	60	12	26	3
Enhancers				
EMS	13	1	38	2
X ray	13	8	29	9

Summary of the number of homozygous viable and homozygous lethal second and third chromosome modifiers obtained from the EMS and X-ray mutageneses. Not included are three X-ray-generated suppressor mutations likely to be translocations to the *Y*, for which it was not possible to know whether they were homozygous viable or lethal.

in JAK kinase), consistent with a role for Drosophila Jak in cell proliferation and that Cyclin D-Cdk4 and Cyclin E-Cdk2 bind and regulate STAT92E protein stability (Chen *et al.* 2003); *fat* (encoding an atypical Cadherin involved in planar polarity); *expanded* (encoding a FERM domain protein involved in actin remodeling); and the unidentified *air7*, *air10*, and *air16* (Gateff *et al.* 1996; de Lorenzo *et al.* 1999). The Drosophila E-cadherin gene, *shotgun* (*shg*; Tepass *et al.* 1996; Uemura *et al.* 1996), when halved in dosage, was also shown to slightly suppress $DmcycE^{JP}$. In contrast, *lethal* (*3*) *malignant brain tumor* [*l(3)mbt*] and *hyperplastic discs* (*hyd*; Gateff *et al.* 1996; de Lorenzo *et al.* 1999), which were considered candidates for the regions 97A–98A2 and 85D8–85E13, respectively (Table 1), did not modify the $DmcycE^{JP}$ phenotype when specific alleles were tested (Table 2; data not shown). Taken together these data suggest that there are specific pathways that show rate-limiting effects on Cyclin E and thereby entry into S phase, in the eye imaginal disc.

Identification of *cyclin E* interactors using a mutagenic dominant modifier screen: As described above, screening for dominant genetic modifiers of $DmcycE^{JP}$ using deficiencies and candidate gene approaches has revealed some interesting interactors. However, this approach is limited in that the deficiencies may remove more than one modifier, confounding the identification of interacting genes. For these reasons, an unbiased genetic screen for $DmcycE^{JP}$ modifiers using mutagenized flies was carried out, to generate specific modifier mutations that could be further characterized. To randomly generate mutations that could then be examined for their effect on the $DmcycE^{JP}$ phenotype, we utilized X-ray mutagenesis, which causes deletions and chromosomal rearrangements (Sankaranarayanan and Sobels 1976) that are expected to aid in the identification of the modifier, and EMS mutagenesis, which causes nucleotide substitutions resulting in missense or nonsense mutations (Lifschytz and Falk 1968). For the X-ray mutagenesis, 39,234 F_1 flies were screened for modification of the $DmcycE^{JP}$ rough eye phenotype and stocks of 104 suppressors and 59 enhancers that consistently modified the $DmcycE^{JP}$ phenotype on the second or third chromosomes were generated (summarized in Table 3). For the EMS mutagenesis a total of 15,049 F_1 flies were screened and 29 suppressors and 54 enhancer mutations on the second or third chromosomes were isolated (Table 3).

***DmcycE*^{JP} suppressor complementation groups:** For the second chromosome homozygous lethal suppressors, complementation analysis revealed that there were 10 complementation groups containing more than one allele, as well as many with single alleles (Tables 4 and 5; and data not shown). In addition, these stocks were crossed to a number of alleles on the second chromosome identified in the screens for enhancers of the eye phenotypes generated by overexpression of *cyclin E* or *E2F1/Dp* (Staehling-Hampton *et al.* 1999; Lane *et al.* 2000). This analysis revealed that *62S9* was allelic to $E(sev\text{-}cycE)^{e93}$ (and was termed group *2.11*). Further analysis revealed that some members of group *2.6* contained a second lethal allele that was distinct from the lethal common to group *2.6* members, forming two new groups, *2.12* (containing the *2.6* allele, *42S13*, and a single allele *22S9*) and *2.13* (containing the *2.6* alleles *42S14* and *66S4* and the *2.7* allele *55S2*). Thus there were a total of 13 second chromosome suppressor groups with multiple members. For the third chromosome suppressors, complementation analysis revealed that there were 5 groups containing >1 allele, and there were many single alleles (Tables 4 and 5). Groups *3.3* and *3.4*, however, cannot truly be considered as groups with more than one allele as there were only two members in each and they both contained a common member, *65S55*, which appears to contain a large deletion. The suppression of the $DmcycE^{JP}$ adult eye phenotype by representatives of the identified suppressor groups is shown in Figure 1.

TABLE 4

Summary of identified suppressors

Group	Alleles	Cytological location	Stage lethal	Identified genes
		Second chromosome		
2.1	*23S9*, *27S3*, *E2S31*, *E6S2*	Genetically linked to *aristaless* (21C) FTC: *Df(2L)net-PMF* C: *Df(2L)al* 21A1–B8	Third larval instar	*lethal-2-giant larvae* (*lgl*)
2.2	*28S2*, *38S4*, *39S2*	Genetically between *cinnabar* (43E) and *brown* (59E) FTC: *Df(2R)trix* C: *Df(2R)3072r* and *Df(2R)CX1* 51A1–5	Embryonic	*phyllopod*
2.5	*42S11*, *58S12*	FTC: *Df(2R)nap9* and *Df(2R)ST1* C: *Df(2R)nap1* and *In(2R)pk78s* 42B3–42C7	Pupal	*l(2)04524 dEB1*
2.11	*62S9*, *E(sev-cycE)*^{e93}	FTC: *Df(2R)Jp1* and *Df(2R)XTE*^{18} C: *Df(2R)03072* and *Df(2R)Jp4* 51D3–51F13	Embryonic	*l(2)01288 scab* (α-*integrin*)
2.12	*42S13*, *22S9*	FTC: *Df(2L)TW137*, *Df(2L)H20*, and *Df(2L)M36F-55* C: *Df(2L)TW50* and *Df(2L)TW3* 36D1–E4	Larval/pupal	*cadN*[a]
		Third chromosome		
3.5	*25S14*, *E6S8*	FTC: *Df(3R)brm11*[b] C: *Df(3R)BK10* and *Df(3R)st-f13* 71F1–72D1	Embryo	*brahma*
2S1		FTC: *Df(3L)emc5*[c] C: *Df(3L)emc-E12*, *Df(3L)Ar11*, and *Df(3L)R*^{G5} 61E–62A8	Before third larval instar	*trio* (*Rac GEF*)
35S1		Genetically between *stripe* (86D1) and *curled* (90F7) FTC: *Df(3R)sbd-105*[d] C: *Df(3R)ea* and *Df(3R)PO4* 89A11–89B10	Embryo	*moira*
43S2		Genetically left of *scarlet* (73A4) FTC: *Df(3L)st-f13*,[d] *Df(3L)st-g24*,[d] and *Df(3L)th102*[d] C: *Df(3L)st-b11* and *Df(3L)brm11* 72D1–72D10	Pupal	*l(3)72Dk zn72D*
63S15		FTC: *Df(3R)T1*^{P},[b] *Df(3R)Tl-X*, and *Df(3R)Tl-I* C: *Df(3R)XTAI* and *Df(3R)3450* 97B–97D2	Third larval instar	*scribble*
65S19		Genetically between *veinlet* (62A) and *thread* (72D) FTC: *Df(3L)fz-M21*[d] C: *Df(3L)fz-GR3b* and *Df(3L)BK10* 70D4–71C3	Semilethal	*trithorax-like*

Underlined alleles are members of more than one group. FTC, failed to complement; C, complemented.
[a] Not confirmed by testing specific allele for suppression of *DmcycE*^{JP}.
[b] Deficiency also suppressed *DmcycE*^{JP} (Table 1 and data not shown).
[c] Deficiency did not suppress and in fact enhanced *DmcycE*^{JP} (see Table 1).
[d] Deficiency did not suppress *DmcycE*^{JP} (data not shown).

Complementation crosses revealed genetic interactions between many *DmcycE*^{JP} modifiers. Some alleles when *trans*-heterozygous showed reduced numbers and/or a striking phenotype characterized by rough eyes, held-out wings, and poor viability [the *r*ough *e*yes and *h*eld-*o*ut *w*ings (Rehow) phenotype] and in some cases the *trans*-heterozygous females were sterile. The Rehow phenotype occurred between the severe group

TABLE 5

Summary of unidentified suppressors

Group	Alleles	Cytological location	Effect on $DmcycE^{JP}$ eye disc S phases	Stage lethal
		Second chromosome		
2.3	*59S16*, *65S12*	Genetically between *black* (34D) and *cinnabar* (43E) FTC: *Df(2L)TW137*, *Df(2L)TW50*, *Df(2L)E71*, *Df(2L)TW3* and *Df(2L)OD15* C: *Df(2L)H20* and *Df(2L)PR-A16* 36F7–37B8	Increased	Pupal
2.4	*26S8*, *57S6*, *59S3*	Genetically between *black* (34D) and *cinnabar* (43E) FTC: *In(2R)bw*^{VDe2L} and *Df(2R)nap1* C: *Df(2R)nap9* 41D2–42A2	Increased	Postembryonic Before third larval instar
2.6	*41S1*, *42S7*, *42S13*, *42S14*, *65S4*, *66S4*, *67S7*, *E3S17*, *E3S18*, *E3S31*	Unknown	Increased	Larval/pupal
2.7	*55S2*, *64S19*, *65S39* (*14S3*, *57S1*, *65S23*, *E10S15*)	FTC: *Df(2R)M60E* C: *Df(2R)ES1* 60E2–8	Increased	Before third instar larvae
2.8	*E6S4*,[a] *E6S19*	FTC: *Df(2L)Dwee1*$^{\Delta 5}$ and *Df(2L)spd*^{j2} C: *Df(2L)Dwee1*^{w05}, *Df(2L)J-H*, and *Df(2L)E110* 27B2–C3	Increased	Larval lethal
2.9	*25S11*, *E1S4*	FTC: *Df(2R)X58-12* and *Df(2R)X58-8* C: *Df(2R)X58-7*, *Df(2R)59AD*, and *Df(2R)pu-D17* 58E4–59A	Increased	Postembryonic Before third larval instar
2.10	*65S5*, *65S13*, *E10S34*[b]	FTC: *In(2R)bw*^{VDe2l}*Cy*^{R} C: *Df(2R)M41A4* and *Df(2R)nap9* 41A–41E1	Increased	Embryonic
2.13	*42S14*, *66S4*, *55S2*	FTC: *Df(2R)M60E* C: *Df(2R)ES1* 60E2–8	Increased	Larval/pupal
		Third chromosome		
3.1	*19S5*, *24SX*, *58S5*, *62S2*	Genetically left of *thread* (72D) FTC: *Df(3L)81k19*[d] C: *Df(3L)st-b11* and *Df(3L)W10* 73D–74F (male recombination mapping to 74B1–B4)	Increased	Larval
3.2	*41S13*, *44S18*, *59S26*, *65S1*, *E6S25*, *E8S13*	FTC: *Df(3L)lxd6*, *Df(3L)vin5*,[d] and *Df(3L)vin2*[d] C: *Df(3L)vin7* and *Df(3L)AC1* 68A9–68B3	Increased	Pupal
3.3	*34S3*, *65S55*	Genetically between *veinlet* (62A) and *hairy* (66D10) 62A–66D10[e]	Increased	Larval
3.4[c]	*1S2*	Genetically left of *scarlet* (73A3)	Increased	Before third instar larval
	65S55	FTC: *Df(3L)29A6*[f] and *Df(3L)Rd1-2*[d] C: *Df(3L)AC1* 66F5 Breakpoint at 66F		

(*continued*)

TABLE 5

(Continued)

Group	Alleles	Cytological location	Effect on *DmcycE*^{JP} eye disc S phases	Stage lethal
		Third chromosome, single alleles		
1S3		Genetically right of *ebony* (93D2) FTC: *Tp(3;Y)J55* C: *Df(3R)T1-P*, *Df(3R)D605*, *Df(3R)3459*, *Df(3R)Dr-rv1*, *Df(3R)L127*, and *Df(3R)B81* 98A–100B (98A5–98E3) Breakpoint at 98C	Increased	Before third instar larval/pupal
13S1		Genetically right of *curled* (86D1) FTC: *Df(3R)Dl-BX12*[f] C: *Df(3R)Cha7*, *Df(3R)KX18*, and *Df(3R)FX3* 91F5–91F11	ND	Before third instar larval
20S1		Genetically to the left of *hairy* (66D10) 61A–66D10[e] Breakpoint at 63E	Increased	Before third instar larval
42S12		Genetically to the left of *hairy* 66D10) 61A–66D10[e] Breakpoint at 62E/F	Increased	Before third instar larval
42S33		Genetically to the left of *thread* (72D) 61A–72D[e]	Increased	Second instar larval
43S1		Unknown	Increased	Before third instar larval
47S8		Unknown	Increased	Before third instar larval
59S9		Genetically between *veinlet* (62A) and *thread* (72D) FTC: *Df(3L)R*^{G7}[g] C: *Df(3L)Aprt-1* and *Df(3L)M21* 62D2–62F5 Breakpoint at 62B	Increased	Semilethal
59S18		FTC: *Df(3L)R*^{G5}[f] C: *Df(3L)Aprt-1*, *Df(3L)Aprt-32*, and *Df(3L)R*^{G7} 62A10–62B1	Increased	Before third instar larval
68S10		FTC: *Df(3R)3-4*[f] C: *Df(3R)110* and *Df(3R)e1025-14* 82F3–82F10	Increased	Before third instar
E9S1		Genetically between *veinlet* (62A) and *thread* (72D) 62A–72D[e]	Increased	Pupal

Underlined alleles are members of more than one group. *55S2* is a member of *2.7* and *2.13*, but other *2.7* alleles complement the *2.13* alleles, *42S14* and *66S4*. Weak alleles are in parentheses. These gave escapers that showed the Rehow phenotype with other *2.7* alleles, although they failed to complement *Df(2R)M60E*. FTC, failed to complement; C, complemented; ND, not determined.

[a] *E6S4* also contains another lethal at 29D1–2 to 30C4–D1.

[b] All three alleles failed to complement each other, but *65S13* and *E10S34* were not completely lethal over the deficiency and gave rise to escapers with rough eyes and wing defects.

[c] Mapping data are for *1S2*.

[d] Deficiency also suppressed *DmcycE*^{JP} (Table 1 and data not shown).

[e] The given cytological interval was determined only by genetic mapping of the lethal. No deficiencies uncovering this mutant were identified by deficiency mapping.

[f] Deficiency did not suppress *DmcycE*^{JP} (data not shown).

[g] Deficiency did not suppress but rather enhanced *DmcycE*^{JP} (see Table 1).

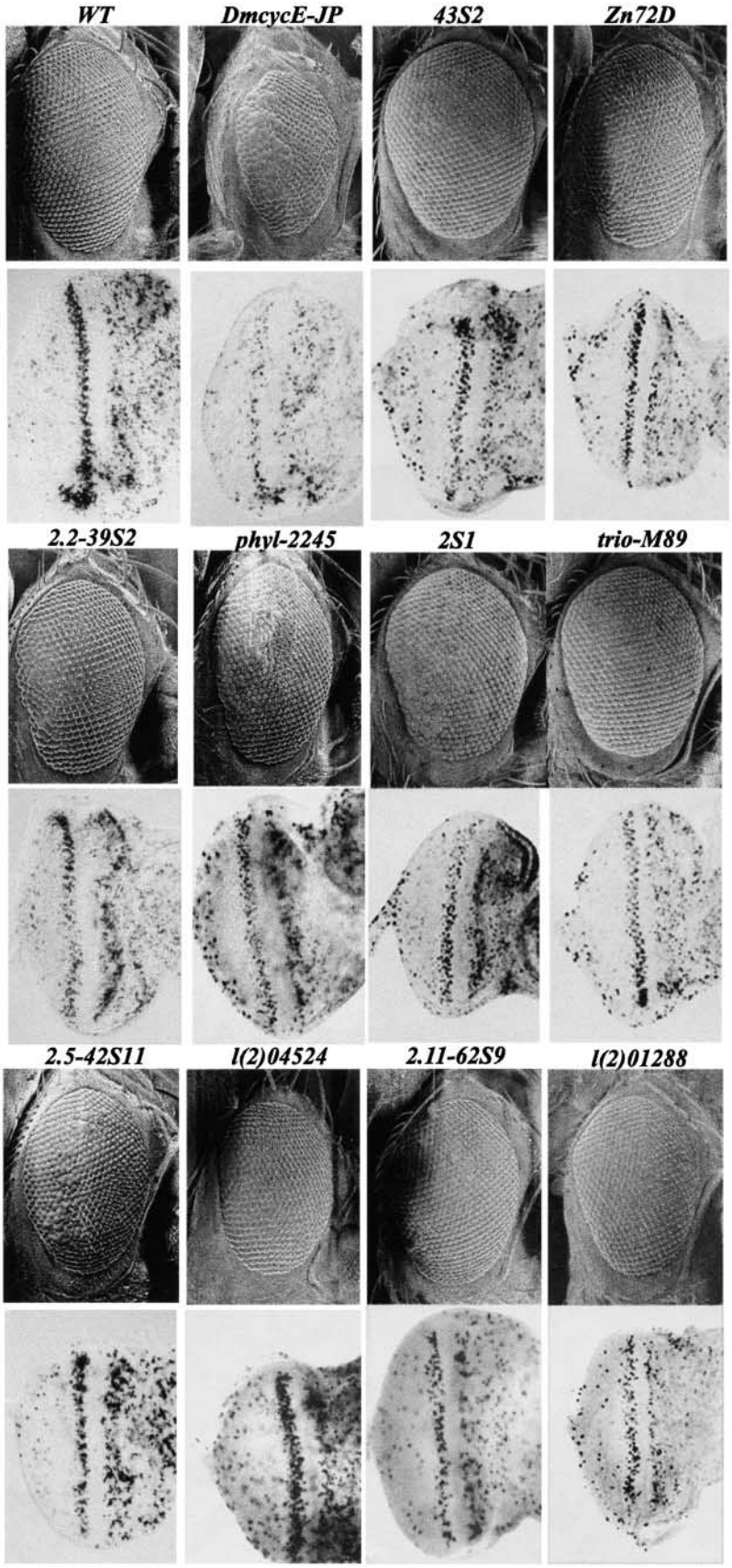

FIGURE 1.—The identified dominant suppressors of *DmcycE^{JP}*: scanning electron-micrographs of adult eyes and BrdU labeling of eye imaginal discs from *DmcycE^{JP}* individuals heterozygous for the identified suppressor alleles. Genotypes are as indicated: wild type (WT); *DmcycE^{JP}*; *DmcycE^{JP}*; *43S2*/+; *DmcycE^{JP}*; *zn72D*/+; *DmcycE^{JP}*, *2.2-39S2*/+; *DmcycE^{JP}*, *phyl^{2245}*/+; *DmcycE^{JP}*; *2S1*/+; *DmcycE^{JP}*; *trio^{M89}*/+; *DmcycE^{JP}*, *2.5-42S11*/+; *DmcycE^{JP}*, *dEB1-l(2)04524*/+; *DmcycE^{JP}*, *2.11-62S9*/+; and *DmcycE^{JP}*, *2.11-l(2)01288*/+.

2.7 alleles (*55S2*, *64S19*, and *65S39*) and the weak *2.7* alleles (*65S23*, *E10S15)* or the single alleles *19S3*, *40S5*, *42S3*, *64S10*, *61S10*, or *62S9* (group *2.11*). These single alleles also showed the Rehow phenotype when crossed with each other. The *trans*-heterozygous Rehow phenotype of *61S10* and the severe *2.7* alleles was dependent on the presence of the *DmcycE^{JP}* mutant, since it was observed only when *DmcycE^{JP}* was homozygous. For *55S2*, *64S19*, *65S39*, *19S3*, *40S5*, *42S3*, *62S9*, *65S23*, *E10S15*, or *64S10*, the Rehow phenotype occurred independent of *DmcycE^{JP}* homozygosity (in the background of *DmcycE^{JP}*/+). Crosses between the third chromosome single-allele suppressors; *47S8* and *59S9*, *20S1*, *63S15*, or *65S19*; also gave rise to the Rehow phenotype. In these cases, genetic and deficiency mapping data suggest that none of these alleles are weak alleles of the same complementation group.

Basic characterization was then carried out on second chromosome multimember complementation groups and most of the third chromosome suppressors (summarized in Tables 4 and 5). We determined whether the suppression of *DmcycE^{JP}* was occurring at the level of S phases during eye development (shown for representatives of the identified suppressors in Figure 1). In all cases examined, there was a significant increase in the size of the eye disc as well as in the number of S phases in the anterior and the post-MF S-phase band. Thus all suppressors tested act to suppress *DmcycE^{JP}* by increasing S phases in the normal pattern. The stage of lethality of the homozygous modifier mutation was also determined by counting the number of hatched embryos and examining whether homozygous third instar larvae were present (Tables 4 and 5). This analysis led to the observation that group *2.1* and *63S15* homozygotes died as overgrown larvae, a phenotype that occurs with Drosophila neoplastic tumor suppressors (Gateff *et al.* 1996; de Lorenzo *et al.* 1999; see below).

Mapping and identification of *DmcycE^{JP}* suppressors: The cytological location of the lethal mutation for the complementation groups and some of the single alleles was determined by crossing suppressors to the deficiency collection (Bloomington Stock Center). In addition, a crude map position was determined for most of the third chromosome interactors and some of the second chromosome interactors by genetic mapping of the *DmcycE^{JP}* suppressor mutation. In all cases tested, the map location of the suppressor by genetic mapping was consistent with the map location of the lethal by deficiency mapping. In some cases, chromosome cytology was examined to map aberrations (Tables 4 and 5). Knowledge of the location of the modifier gene then enabled likely candidate genes to be investigated by testing mutant alleles, where available, for failure to complement the modifier mutant.

This strategy enabled the identification of 5 of the 13 second chromosome (*2.1*, *2.2*, *2.5*, *2.11*, and *2.12*) and 6 of the 20 third chromosome suppressors (*3.5*, *2S1*, *35S1*, *43S2*, *63S15*, and *65S19*; summarized in Table 4). Of the remaining groups, although map positions were well defined for 7 of 13 of the second (*2.3*, *2.4*, *2.7*, *2.8*, *2.9*, *2.10*, *2.13*) and 8 of 20 third chromosome suppressor groups (*3.1*, *3.2*, *3.4*, *1S3*, *13S1*, *59S9*, *59S18*, *68S10*), and available candidate gene alleles were tested for each of the suppressor genes, the identity of the suppressors is not yet known (Tables 5 and 7). In these cases, it is likely that these suppressor mutations define novel genes. For two of the suppressor groups (*2.6* and *3.3*) and five of the single alleles (*20S1*, *42S12*, *42S33*, *43S1*, *47S8*) a precise location for the suppressor was not determined, since none of the available deficiencies failed to complement the suppressor. In these cases the lethal mutation must map to a region not covered by the deficiency collection. A brief description of the identification of the more precisely localized suppressors is detailed below and summarized in Tables 4 and 5.

The identified suppressors: By complementation tests to known gene alleles the identities of five second chromosome suppressors (*2.1*, *2.2, 2.5*, *2.11*, *2.12*) and six third chromosome suppressors (*3.5*, *2S1*, *35S1*, *43S2*, *63S15*, *65S19*) were revealed. These suppressor genes fall into the functional groups of chromatin remodeling and transcription factors (four genes), signaling (two genes), cytoskeletal (one gene), cell adhesion (two genes), and neoplastic tumor suppressors (two genes). Specific details on the verification and characterization of these suppressors are discussed under these functional groupings (summarized in Table 4).

Chromatin remodeling and transcription factor genes: *3.5 (Brahma): 3.5* was mapped to 71F1–72D1 (Table 4) and alleles of *brahma*, a *SWI2* homolog, encoding a component of the SWI/SNF chromatin-remodeling complex (Papoulas *et al.* 1998), failed to complement both *3.5* alleles. Furthermore, *Df(3R)brm11* (71F1–4; 72D1–10) was identified as a dominant suppressor of *DmcycE^{JP}* in the screen of third chromosome deficiencies (Table 1). Consistent with suppressor *3.5* being *brahma*, we showed that previously isolated alleles of *brahma* also dominantly suppressed *DmcycE^{JP}* (Brumby *et al.* 2002).

35S1 (Moira): 35S1 was mapped to 89A11–89B10 (Table 4). Candidate mutants in this region were tested for allelism with *35S1*, and alleles in *moira*, a *SWI3* (*BAP155)* homolog, failed to complement *35S1.* Consistent with the suppressor *35S1* being *moira*, we demonstrated that previously isolated alleles of *moira* also dominantly suppressed *DmcycE^{JP}* (Brumby *et al.* 2002).

Brahma and Moira are components of the Drosophila Brahma (SWI/SNF-related) chromatin remodeling complex (Papoulas *et al.* 1998), which has been shown to play a role in negatively regulating S phase (Staehling-Hampton *et al.* 1999; Harbour and Dean 2000). Consistent with this notion, alleles of other Brahma complex genes, *snr1* and *osa*, as well as a deficiency that removes the *brahma-associated protein 60* (*BAP60*) or *BAP111*, dom-

inantly suppress the *DmcycE^{JP}* phenotype (Brumby *et al.* 2002; Table 1).

65S19 (Trithorax-like): 65S19 was only semilethal; however, genetic and deficiency mapping was still possible, and *65S19* was located to 70D4–71C3 (Table 4). Complementation tests of candidate genes in the region revealed that *Trithorax-like* (*Trl*) was allelic to *65S19*. Consistent with this, previously characterized alleles of *Trl* also dominantly suppressed *DmcycE^{JP}* (Brumby *et al.* 2002).

43S2[l(3)72Dk (zn72D)]: 43S2 was localized to 72D1–72D10 (Table 4) and complementation tests of mutations in the 72D1–10 region revealed that *In(3)Taf4$^{XS\text{-}2884}$*, an inversion affecting expression of *Taf4* (*Taf110*) and *Zn72D* (Sauer *et al.* 1996), failed to complement *43S2*. A specific EMS allele of *Taf4*, *l(3)72Dj*, however, complemented *43S2*, suggesting that *43S2* is most likely allelic to *zn72D* (*CG5215*). Indeed, another EMS allele in the region, *l(3)72Dk*, which failed to complement *In(3)Taf4$^{XS\text{-}2884}$*, also failed to complement *43S2*, suggesting that *l(3)72Dk* is an allele of *zn72D*. The *zn72D* gene encodes a zinc finger protein, but has not been characterized. In an attempt to verify the identity of *43S2* suppression as being due to a mutation of *zn72D*, *l(3)72Dk* was crossed into the *DmcycE^{JP}* background. However, *l(3)72Dk* did not suppress the *DmcycE^{JP}* adult eye phenotype or the S-phase defect of *DmcycE^{JP}* eye discs as effectively as *43S2* did (Figure 1), which may be due to *l(3)72Dk* being a weaker allele than *43S2*. Molecular characterization of the *43S2* and *l(3)72Dk* lesion will be required to confirm this. Interestingly, *Zn72D* was identified in a differential expression screen as a gene expressed specifically in the differentiating region of the eye disc (Jasper *et al.* 2002), consistent with a role for Zn72D in cell cycle arrest or differentiation.

Signaling pathway genes: *2.2 (phyllopod): 2.2* was localized to 51A1–51A5 (Table 4). Consistent with this, *Df(2R)trix* (51A1–2; 51B6) dominantly suppressed the *DmcycE^{JP}* rough eye phenotype (data not shown). Mutations and *P* alleles within the 51A region were tested for allelism with *2.2* alleles, revealing that a null allele of *phyllopod*, *phyl^{2245}*, failed to complement all three *S(DmcycE^{JP}) 2.2* alleles. To verify that *2.2* was indeed *phyl*, previously identified *phyl* alleles (*2245* and *2366*) were tested and shown to dominantly suppress the rough eye phenotype and the S-phase defects of *DmcycE^{JP}* (Figure 1; M. Coombe, L. Quinn, R. Dickins, J. Secombe and H. Richardson, unpublished results). These data are consistent with the mutation of *phyl* being responsible for the observed suppression of *DmcycE^{JP}* by the *2.2* alleles.

Phyl expression is induced by the Sevenless receptor tyrosine kinase signaling pathway and is a rate-limiting component in R7 photoreceptor cell differentiation in the eye imaginal disc, but also has other roles in neural differentiation during development (Dickson 1998). Phyl is a pioneer protein (containing no homology to other known proteins) that functions with the Ring finger protein Seven in absentia (Sina) and the F-box protein Ebi, to bind to and target the two isoforms of the neural differentiation inhibitor, Tramtrack (Ttk69 and Ttk88) and probably other proteins for destruction by the ubiquitin/proteosome pathway, allowing neural cell differentiation (Li *et al.* 1997; Tang *et al.* 1997; Boulton *et al.* 2000). Consistent with this, homozygous viable mutants in *sina* (*sina^{1}*) strongly suppressed the *DmcycE^{JP}* adult rough eye and S-phase defects, while a stronger *sina* allele (*sina^{2}*) showed weak dominant suppression (M. Coombe, L. Quinn, R. Dickins, J. Secombe and H. Richardson, unpublished results). However, a deficiency removing *sina* showed strong dominant suppression of *DmcycE^{JP}* (Table 1). This deficiency removes a *sina*-related gene (*sina-h*), located adjacent to *sina*, as well as *Abl*, which has been shown to dominantly suppress *DmcycE^{JP}* (see below). Consistent with the involvement of the Sina complex in negative regulation of G1-S, *ebi* alleles have been shown to dominantly suppress *DmcycE^{JP}* (Boulton *et al.* 2000). The mechanism by which the Sina complex acts to regulate G1-S does not involve targeting Cyclin E or E2F for ubiquitin-dependent degradation (Boulton *et al.* 2000) and remains to be determined.

2S1 (trio): 2S1 was mapped to 61E–62A8 (Table 4) and by crosses to mutations within the region it was revealed that *trio* [encoding a Rac guanine nucleotide exchange factor (Rac-GEF; Bateman *et al.* 2000)] failed to complement *2S1*. To confirm this interaction, a previously isolated allele of *trio* (*trio^{M89}*) was crossed into the *DmcycE^{JP}* background. *trio^{M89}* was shown to dominantly suppress the *DmcycE^{JP}* rough eye phenotype and S-phase defect (Figure 1). Rac-GEFs are involved in the activation of Rac family GTPases, which have roles in actin cytoskeletal remodeling (Blanchard 2000). In mammalian cells, Rac can lead to repression of Rho activity (Sander *et al.* 1999), and therefore mutation of *trio* may lead to higher levels of Rho activity. Rho activation in mammalian cells has been shown to promote cell cycle progression by leading to downregulation of the Cyclin/Cdk inhibitors p21 and p27 (Aznar and Lacal 2001; Pruitt and Der 2001; Sahai and Marshall 2002). *trio* has been shown to genetically interact with *Abl*, encoding a nonreceptor tyrosine kinase also involved in actin cytoskeleton remodeling (Luo 2000). Consistent with this, the deficiency removing *Abl* (73A3; 74F) dominantly suppressed the *DmcycE^{JP}* rough eye phenotype; however, this deficiency also removes *sina*, *sina-h* (see above), and the Abl pathway gene, *Disabled* (*Dab*). The *Abl* alleles *Abl^{04674}* and *Abl^{1}* were then tested and shown to also suppress the *DmcycE^{JP}* rough eye phenotype (not shown). The precise mechanism by which reducing the dosage of *trio* and *Abl* leads *DmcycE^{JP}* suppression remains to be determined.

Cytoskeletal genes: *2.5 (dEB1): 2.5* was localized to

42B3–42C7 (Table 4). *2.5^{58S12}* was also lethal over the adjacent deficiency, *Df(2R)nap1* (41D2–E1; 42B1–3), indicating that this allele is a deficiency or rearrangement that affects a larger region than *2.5^{42S11}*. *S(DmcycE^{JP})2.5^{42S11}* was crossed to *P*-element alleles available in the region and *l(2)04524*, was found to be semilethal in combination with *2.5^{42S11}*. The few escaper flies, *trans*-heterozygous for *2.5^{42S11}* and *l(2)04524*, did not have any gross abnormalities, but generally died within a few days of eclosing, and the females were sterile. *l(2)04524* is inserted within the 5′-UTR of the Drosophila homolog of the *EB1* gene (BDGP). *dEB1* encodes a cytoskeleta1 protein that binds to microtubules and plays an important role in adherens junction integrity and cell polarity (Lu *et al.* 2001; Rogers *et al.* 2002). EB1 was identified in mammalian cells as a binding partner of the adenomatous polyposis coli (APC) colon cancer tumor suppressor (Su *et al.* 1995); however, Drosophila APC1 and APC2 both lack the EB1-binding domain. Consistent with the identity of *2.5* being *dEB1, l(2)04524* and the EMS *dEB1* alleles, *dEB1^{5}* (*1DL)* and *dEB1^{6}* (*GJ63/9*) (obtained from J. Roote), dominantly suppressed *DmcycE^{JP}* rough eye and S-phase defects (Figure 1 and data not shown). Moreover *2.5^{42S11}* and *l(2)04524* disrupt *dEB1* transcription (D. Coates, L. Quinn, R. Dickins, J. Secombe, A. Brumby and H. Richardson, unpublished results). How the EB1 microtubule protein is involved in G1-S regulation remains to be determined.

Cell adhesion genes: *2.11 (scab) (α-Integrin):* Group *2.11* was defined by *S(DmcycE^{JP})62S9* from this screen and *E(sev-cycE)^{e93}* was from the Lane *et al.* (2000) genetic screen (see above). *2.11* was mapped to the region 51D3–51F13 (Table 4), and by testing mutations within this region, it was revealed that the *P* allele, *l(2)01288*, failed to complement both *2.11* alleles. The insertion point of *l(2)01288* has been defined (BDGP) and disrupts the *scab* gene, encoding an α-integrin, αPS3, thought to play a role in tissue morphogenesis (Stark *et al.* 1997). To further confirm that *2.11* is allelic to *scab*, previously identified EMS-derived alleles of *scab* (*scb^{1}* and *scb^{2}*) were tested and shown to also fail to complement *2.11* alleles. Consistent with the suppressing gene being *scab*, *l(2)01288*, *scb*^{1}, and *scb*^{2} were recombined onto the *DmcycE^{JP}* and were shown to also suppress the rough eye phenotype and the S-phase defect of *DmcycE^{JP}* (Figure 1 and data not shown). In mammalian cells, integrins in association with the extracellular matrix have a well-established role in promoting anchorage-dependent cell proliferation (Danen and Yamada 2001). However, recent studies have shown that integrins can also inhibit G1-S progression (Hazlehurst *et al.* 2000; Mettouchi *et al.* 2001). Our identification of *scab* in the *DmcycE^{JP}* genetic screen suggests that in Drosophila integrins also act as negative regulators of G1-S.

2.12 (CadN): 2.12 alleles *42S13* (also an allele of group *2-6*) and *22S9* (Figure 1 and data not shown) were mapped to 36D1–36E4 (Table 4). Mutations and *P* alleles in the region were tested by complementation analysis, revealing that an allele of *CadN* (*CadN^{M12}*) failed to complement both *2.12* alleles. *CadN* encodes a cadherin-like transmembrane protein (Lee *et al.* 2001; Iwai *et al.* 2002) that can bind to α-catenin and β-catenin (Armadillo), components of the adherens junction (Perez-Moreno *et al.* 2003). In mammalian cells, downregulation of N-Cadherin leads to upregulation of G1 Cyclin activity (Charrasse *et al.* 2002). Due to the close location of *CadN* and *DmcycE*, it was not possible to obtain a recombinant of the *CadN* allele with *DmcycE^{JP}* to confirm that *CadN* exhibits the same modifier effect as *S*(*DmcycE^{JP}*) *2.12*.

Cytoarchitectural tumor suppressor genes: *2.1 [lethal-(2)-giant larvae]: 2.1* was localized to 21A1–21B7–8 by deficiency mapping (Table 4). The mapping of *2.1* was initially confounded by the fact that two deficiencies in the deficiency kit, *Df(2L)Prl* (32F1–3; 33F1–2) and *Df(2L)J39* (31D1–11; 32D1–E5), also contained lesions in the 21A region and therefore failed to complement *2.1*. The localization of *2.1* was confirmed by genetic mapping of *2.1* alleles, which indicated that the lethal mapped to the left of *UbcD1* (32A4–5) and close to *al* (21C2–4). Since *2.1* homozygous mutants die as giant larvae, an allele of the *lethal-(2)-giant-larvae* (*lgl*) gene, which also gives giant larvae and is localized at 21A, was tested for complementation of *2.1* alleles and failed to complement, whereas mutations in other genes in this region that have been identified as negative cell cycle regulators in previous screens, *spen* (*poc*; Staehling-Hampton *et al.* 1999; Lane *et al.* 2000) and *net* (I. Harriharan, personal communication), both complemented *2.1* alleles. Taken together these data suggest that *lgl* corresponds to *2.1*. To confirm that a lesion in *lgl* suppresses the *DmcycE^{JP}* phenotype, a null allele of *lgl* (*lgl^{4}*) was tested for suppression of *DmcycE^{JP}*. However, *lgl^{4}* did not suppress the S-phase defect or the rough eye phenotype of *DmcycE^{JP}* to the same extent as *2.1* alleles did (Figure 2; and data not shown). However, halving the dosage of *2.1* alleles resulted in a greater increase in Cyclin E protein levels in *DmcycE^{JP}* eye discs than halving the dosage of *lgl^{4}/+* (Figure 3). It is possible that additional mutations in the *lgl^{4}* background may account for its poorer ability to dominantly suppress *DmcycE^{JP}* compared with *2.1* alleles. Consistent with *lgl* mutations being responsible for the suppression of *DmcycE^{JP}*, *lgl-2.1* and other *lgl* mutant clones in the eye imaginal disc showed ectopic expression of Cyclin E, which could be suppressed by expression of *lgl* using a *UAS-lgl* transgene (N. Amin, A. Brumby, J. Secombe and H. Richardson, unpublished results).

63S15 (scribble): 63S15 was localized to 97B–97D2 (Table 4), and consistent with a suppressor mapping in this region, *Df(3R)T1^{P}*, which failed to complement *63S15*, was identified as a suppressor of *DmcycE^{JP}* in the screen of third chromosome deficiencies (Table 1). Cytological analysis of *63S15* showed that there was a lesion in the

97D region involving a translocation to the second chromosome (data not shown). By crosses to *P* alleles in the region, *63S15* was found to be allelic to *l(3)j7b3*, which is located in the first intron of a gene now known as *scribble* (Bilder and Perrimon 2000).

Scribble is a four-PDZ95-Dlg-ZO1 and multi-leucine-rich repeat containing protein localized to septate junctions and required for apical-basal polarity (Bilder and Perrimon 2000; Humbert *et al.* 2003). When homozygous, *63S15*, like *scribble* null alleles, arrest as giant overgrown larvae due to amorphous overgrowth of imaginal discs and brain lobes, which is characteristic of neoplastic tumor suppressor mutants (Gateff *et al.* 1996; de Lorenzo *et al.* 1999; Bilder 2001). To confirm that lesions in *scribble* suppress $DmcycE^{JP}$, the *l(3)j7b3* allele and stronger EMS alleles of *scrib*, $scrib^{1}$ and $scrib^{2}$ (Bilder and Perrimon 2000), were crossed into a $DmcycE^{JP}$ background. The weak *P* allele, *l(3)j7b3*, did not suppress $DmcycE^{JP}$, although mild suppression was observed with $scrib^{1}$ and $scrib^{2}$ alleles, but not as well as with *63S15* (Figure 2 and data not shown). This suggests that *63S15* may be a stronger *scribble* allele than $scrib^{1}$ or $scrib^{2}$. In confirmation that *scribble* alleles suppress the $DmcycE^{JP}$ phenotype, halving the dosage of *scribble* in $DmcycE^{JP}$ eye discs leads to higher levels of Cyclin E (Figure 3 and data not shown) and $scrib^{1}$ and $scrib^{2}$ eye imaginal disc clones show ectopic expression of Cyclin E (Brumby and Richardson 2003).

lgl and *scribble* are neoplastic tumor suppressor genes that together with *discs-large* (*dlg*) act in the same pathway to regulate apical-basal cell polarity (Bilder *et al.* 2000; Humbert *et al.* 2003). Because of this function, we have termed these proteins cytoarchitectural tumor suppressors to highlight their role in cell structure. Consistent with this pathway being important in regulation of G1- to S-phase progression, a deficiency removing *dlg*, $Df(1)v^{N48}$, as well as a specific *dlg* allele (dlg^{6}) showed suppression of $DmcycE^{JP}$ (Tables 1 and 2; Figure 2). Scribble, Dlg, and Lgl have been recently shown to act antagonistically to the Crumbs cell polarity complex (Bilder *et al.* 2003; Tanentzapf and Tepass 2003), and consistent with this, a deficiency removing *crumbs* and a *crumbs* allele (crb^{2}) dominantly enhanced $DmcycE^{JP}$ (Table 1).

***Scribble*-interacting genes:** To determine whether a common pathway is involved in the mechanism by which the $DmcycE^{JP}$ suppressors lead to deregulation of cell proliferation, we analyzed weak *scribble* mutant combinations for a dominant genetic interaction with other genes identified in the *DmcycE* genetic screen (Table 6). The *trans*-heterozygous combination of $scrib^{5}/scrib^{l(3)jB709}$ or $scrib^{1}/scrib^{5}$ results in adults with eye, bristle, and thorax-closure defects (not shown). Reducing the dose of the *lgl* (*27S3*, *E2S31*, and lgl^{4}) showed a strong genetic interaction with the weak *scrib* allele phenotype, resulting in no *scrib* mutant progeny heterozygous for *lgl*. This is consistent with the previous observations that *scribble* mutations exhibit strong genetic interactions with *lgl* and *dlg* in the embryo (Bilder *et al.* 2000). Strikingly, halving the dosage of several other suppressor genes identified in the screen also resulted in very low numbers of *scrib* mutant progeny, most notably with *dEB1* (*2.5*), *phyl* (*2.2*), the αPS3 integrin gene *scab* (*2.11*), the Brahma complex gene *moira*, and to a lesser extent *brahma*, as well as the unidentified *2.3*, *2.4*, and *2.9* genes. The mechanism of these interactions requires further analysis and relies on identifying the *2.3*, *2.4*, and *2.9* genes.

The unidentified suppressors: The map positions for suppressor groups *2.3*, *2.4*, *2.7*, *2.8*, *2.9*, *2.10*, *2.13*, *3.1*, *3.2*, and *3.4* (*1S2*) and the single alleles *1S3*, *13S1*, *59S9*, *59S18*, and *68S10* were defined by genetic and deficiency mapping (Table 5). For the third chromosome suppressors, *3.1*, *3.2*, and *3.4* (*1S2*), the location of a suppressor within the defined region could be confirmed since the corresponding deficiencies dominantly suppressed $DmcycE^{JP}$ (Table 1; and data not shown). However, for *13S1*, *59S9*, *59S18*, and *68S10*, the deficiencies that failed to complement these suppressors did not suppress $DmcycE^{JP}$ (Table 1; and data not shown). For most of the unidentified suppressors complementation tests of all likely mutations and *P* alleles within the respective regions and Southern analysis of candidates have so far failed to identify the affected gene (Table 7); therefore, these suppressor mutations affect novel genes, which will require further analysis to identify. The exception is *2.3*, where there are two candidates (Table 7 and see below). Potential candidates, with links to identified $DmcycE^{JP}$ suppressors and thereby G1-S regulation, were found for many of the unidentified suppressors (see Table 7). Some of these candidates have been tested by complementation tests or Southern analysis and have been ruled out as being affected by the suppressor mutation (Table 7). Details on mapping and potential candidates for *2.3*, *3.1*, *1S3*, and *59S9* are described below. For the details on other unidentified suppressors, see Tables 5 and 7.

2.3 (59S16, 65S12) location (36F7–37B8): While 2.3^{59S16} carries a deletion removing at least six complementation groups within the 36F7–37B8 region, including *l(2)36Fd* and *l(2)37Ac*, 2.3^{65S12} was found to be lethal over the unidentified lethal gene *l(2)36Fd*, but gave ~5% escapers over *l(2)37Ac*. 2.3^{65S12} is therefore likely to be a smaller lesion affecting both of these uncharacterized genes. A recently characterized gene in the 36F region, *hamlet*, which is a transcription factor involved in dendrite morphogenesis (Moore *et al.* 2002), was also tested for allelism with *2.3* and failed to complement 2.3^{59S16} and 2.3^{65S12} but not *l(2)36Fd*. Further analysis is required to determine whether *hamlet* or *l(2)36Fd* corresponds to the *2.3* suppressor.

3.1 (19S5, 24SX, 58S5, 62S2) location [73D–74F (74B1–74C1)]: Consistent with the map position defined by deficiency mapping, chromosome cytology revealed that *58S5* contained a deletion in the 74A–F region,

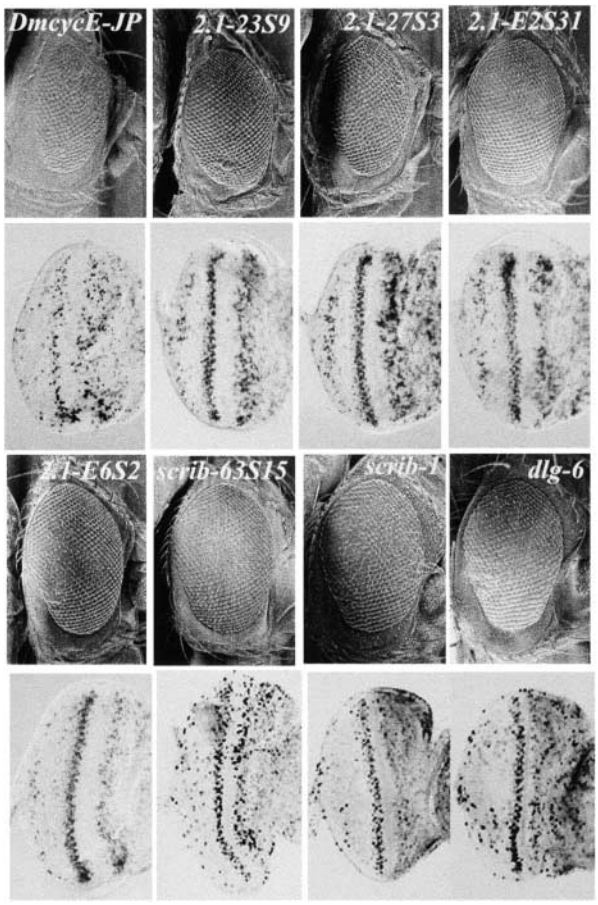

FIGURE 2.—Scanning-electron micrographs of adult eyes and BrdU labeling of eye imaginal discs from *lgl*, *scrib*, or *dlg* heterozygotes in a $DmcycE^{JP}$ background. Genotypes are as indicated: $DmcycE^{JP}$; *2.1-23S9/+*, $DmcycE^{JP}$; *2.1-27S3/+*, $DmcycE^{JP}$; *2.1-E2S31/+*, $DmcycE^{JP}$; *2.1-E6S2/+*, $DmcycE^{JP}$; $DmcycE^{JP}$; *scrib-63S15/+*; $DmcycE^{JP}$; $scrib^{1}$/+; and dlg^{6}/+; $DmcycE^{JP}$.

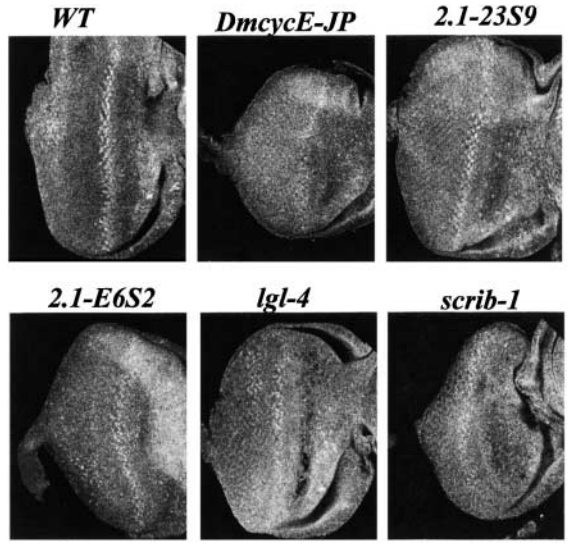

FIGURE 3.—Cyclin E protein levels in eye imaginal discs from third instar larvae. Genotypes are as indicated: wild type (WT); $DmcycE^{JP}$; *2.1-23S9/+*, $DmcycE^{JP}$; *2.1-E6S2/+*, $DmcycE^{JP}$; lgl^{4}/+, $DmcycE^{JP}$; and $DmcycE^{JP}$; $scrib^{1}$/+.

and it failed to complement several lethal alleles in the region. The cadherin-like gene, *CG6445* (*Cad74A*), was considered a candidate, since the cadherin-like protein, Fat, is a tumor suppressor in Drosophila (GATEFF *et al.* 1996; DE LORENZO *et al.* 1999). Southern analysis failed to reveal any alterations in this gene in *3.1* alleles (data not shown). The method of male recombination (SVOBODA *et al.* 1995) was then used to further define the map position of the *3.1* alleles, *19S5* and *24S10* relative to several *P* alleles, revealing that the lethal associated with *3.1* mapped to the right of *blot* (74B1–2) and to the left of *l(3)S070006* (allelic to *l(3)L6750 = frc* at 74B4), *l(3)00073* (74C1–2), and *EIP74EF* (74D2–5). Taken together these data suggest that *3.1* maps between 74B1 and 74B4. A candidate gene within this region, *CG3885*, encodes a Sec3-like protein, a component of the exocyst complex involved in docking at the plasma membrane, which is a function that Lgl has also been implicated in (LEHMAN *et al.* 1999; MUSCH *et al.* 2002).

1S3 location [98A–100B (98A5–98E3)]: Chromosome cytology showed that *1S3* contained a translocation breakpoint at 98C (data not shown). Since there is a hole in the deficiency collection between 98A5 and 98E3, it is likely that *1S3* maps within this region. A candidate in the 98A5–98E3 region was APC1 (encoding the Adenomatous polyposis tumor suppressor; AHMED *et al.* 1998); however, mutations in APC1 (APC^{Q8} and APC^{X1}) complemented *1S3*. Another candidate is *raps* (*pins*), which encodes a protein involved in asymmetric division of neuroblasts and directly interacts with Dlg (PARMENTIER *et al.* 2000; BELLAICHE *et al.* 2001). Further analysis is required to test whether *raps* mutations are allelic to *1S3*.

59S9 location (62D2–62F5): Consistent with this location for *59S9*, cytological analysis revealed a breakpoint at 62B. A possible candidate in this region is *spinophilin* (*neurabin*), encoding an actin-binding scaffold protein, which in mammalian cells is involved in binding to and upregulating Rac and p70-S6K activity (BUCHSBAUM *et al.* 2003). Since another gene involved in Rac activation, *trio*, was identified as a suppressor of $DmcycE^{JP}$ it is possible that *spinophilin* is also a suppressor. Furthermore, Drosophila mutations in *spinophilin* are semilethal (KEEGAN *et al.* 2001), as is *59S9*.

Further analysis is needed to investigate whether the potential candidates for these suppressors, listed above and in Table 7, are disrupted by the suppressor mutations and for the identification of the suppressors.

DISCUSSION

In this study, we have identified genetic interactors of *cyclin E* by screening deficiencies, by testing candidate genes, and through EMS and X-ray mutagenesis screens. This work has led to the identification of many genes

TABLE 6

Genetic interactions with *scribble*—effect of halving the dosage of other *S(DmcycE^{JP})* genes on the viability of hypomorphic *scribble* allele combinations

Mutant allele		Total no. of progeny: x^-/+ or *CyO*/+; $scrib^{1or5}$/*TM6B*	Total no. of progeny: x^-/+; $scrib^1/scrib^5$	% of expected no. of x^-/+ $scrib^1/scrib^5$ progeny[a]
2.1 (*lgl*)	*27S3*	304	0	0
	E2S31	145	0	0
	lgl4	248	0	0
2.2 (*phyl*)	*28S2*	231	0	0
2.3	*65S12*	227	0	0
	36Fd	343	1	2
2.4	*57S6*	235	0	0
	59S3	210	8	25
2.5 (*dEB1*)	*42S11*	147	3	13
2.9	*E1S4*	256	0	0
	25S11	242	0	0
2.11 (*scab*)	*E(sev-cycE)^{e93}*	86	1	8

Mutant allele		Total no. of progeny: x^- $scrib^{J7b3\ or\ 5}$/*TM6B*	Total no. of progeny: x^- $scrib^{J7b3}/scrib^5$	% of expected no. of x^-/+ $scrib^1/scrib^5$ progeny[a]
brm	*2*	401	47	24
mor	*1*	323	13	8
3.1	*19S5*	276	91	66
	24SX	279	58	42

[a] The expected number of $scrib^1/scrib^5$ progeny = total *TM6B* progeny/4 × 0.61 was derived as follows: The control cross of $scrib^1/TM6B \times scrib^5/TM6B$ gave 127 $scrib^1/scrib^5$ out of 627 total progeny, *i.e.*, 61% of the expected number. To control for this, the percentage of the expected number of x^-/+ $scrib^1/scrib^5$ progeny has been adjusted by this factor. The weaker *scrib* allele combination, −/+ $scrib^{J7b3}/scrib^5$, gave expected Mendelian numbers of progeny. The expected number of $scrib^{J7b3}/scrib^5$ progeny = total number of *TM6B* progeny/2.

that when mutated have the ability to dominantly modify the *DmcycE^{JP}* adult rough eye phenotype and S-phase defect in third instar larval eye imaginal discs. In addition to genes already known to be regulators of Drosophila *cyclin E* or G1-S progression, such as *E2F1*; *retinoblastoma* (*Rbf*); *ago* (*cdc4*) encoding a protein involved in Cyclin E degradation (Moberg *et al.* 2001); the EGF receptor pathway genes *Egfr* and *Ras85D*, which act to promote Cyclin E protein accumulation (Prober and Edgar 2000; Brumby and Richardson 2003); and Hh signaling pathway genes, which act to promote *cyclin E* transcription (Duman-Scheel *et al.* 2002); this screen led to the identification of many novel *cyclin E* interactors. This study has mainly concentrated on the suppressors of *DmcycE^{JP}*, although from the deficiency screen and specifically testing candidates, we identified *axin* (an inhibitor of Wg signaling), *rho1*, and *crumbs* as enhancers of *DmcycE^{JP}*, which therefore may act as novel positive regulators of G1-S progression. The suppressors of *DmcycE^{JP}* identified include the following classes: (1) chromatin remodeling genes *brm*, *mor*, *Trl*, or the transcription factor *Zn72D*; (2) signaling pathway genes *phyl*, *sina*, *trio*, *Abl*, *RpS6*, *wg* and Wg pathway effectors *dsh* and *arm*; (3) genes encoding cytoskeletal proteins *dEB1* (encoding a microtubule-binding protein) and *expanded* (encoding a FERM domain cytoskeletal protein and hyperplastic tumor suppressor); (4) genes encoding cell adhesion proteins *scab* (encoding an α-integrin), *cadN* (N-Cadherin), *shg* (E-Cadherin), and *fat* (encoding an atypical-cadherin and hyperplastic tumor suppressor); and (5) cytoarchitectural tumor suppressor genes *scribble*, *lgl*, and *dlg*, required for apical-basal cell polarity and cell proliferation inhibition. While some of these genes (*brm*, *mor*, *expanded*, *fat*, *scribble*, and *lgl*) have been previously shown or implicated to play a role in negatively regulating G1-S (Gateff *et al.* 1996; de Lorenzo *et al.* 1999; Staehling-Hampton *et al.* 1999; Bilder *et al.* 2000), a potential role for *Trl*, *Znf72D*, *phyl*, *sina*, *trio*, *Abl*, *RpS6*, *wg*, *dsh*, *arm*, *dEB1*, *scab*, *cadN*, and *shg* in inhibiting G1-S progression in Drosophila is novel. Further studies are required to determine whether *Abl*, *RpS6*, *wg*, *dsh*, *arm*, and *shg* do indeed suppress *DmcycE^{JP}* by acting at the S-phase level and to understand the mechanism by which these genes act in G1-S regulation. The identification of novel classes of presumptive negative regulators of *cyclin E* or G1-S progression highlights

TABLE 7

Candidates for the unidentified suppressors

Suppressor group	Candidate gene	Gene function	Possible links to cell cycle regulation
2.3	*l(2)36Fd*?	Unknown	Unknown
	hamlet?	Transcription factor in Dendrite morphogenesis (Moore *et al.* 2002)	May be involved in regulating cytoskeletal, cell adhesion or cytoarchitectural tumor suppressor genes
2.4	*Act42A*	Actin 42A	Brahma complex component (Papoulas *et al.* 1998)
	CG12792	WD40 domain	May be involved in proteolysis as is Cdc4/Ago (Moberg *et al.* 2001)
	CG10412	Dbl-related (RhoGEF)	Activator of Rho family proteins and may regulate Rac (Blanchard 2000)
2.7	*CG2727* (*Emp*)[a]	CD36-like	CD36-like proteins encode cell surface signaling proteins that may have a role in adhesion and signaling pathways regulating cell proliferation (Greenwalt *et al.* 1992)
	CG2736[a]	CD36-like	
	CG3829[a]	CD36-like	
	CG3770	Claudin-like	A tight junction protein involved in cell-cell adhesion and may have a role in inhibiting cell proliferation (Michl *et al.* 2003; Tepass *et al.* 2001)
2.8	*wee1*[b]	Cdc2 inhibitor (Campbell *et al.* 1995)	In combination with Cyclin A can drive entry into S phases (Dong *et al.* 1997; Thomas *et al.* 1997)
	neuroligin	Cell adhesion protein	The mammalian homolog binds to Dlg4 (Bolliger *et al.* 2001)
2.9	*Jitterbug* (*Filamin*)[a]	Actin-binding protein	Possibly acts to regulate Rho family members (Sokol and Cooley 2003; Stossel *et al.* 2001)
	moa	Cell adhesion	Possible role in Integrin signalling (Prout *et al.* 1997; Walsh and Brown 1998)
2.10	*p120-catenin*[a]	Adherens junction component	Binds to E-cadherin and regulates Rho in mammalian cells (Aznar and Lacal 2001; Blanchard 2000; Jaffe and Hall 2002)
	Gprk-1	G-protein-coupled receptor protein kinase with a RGS domain	Negative regulator of heterotrimeric G proteins, responsible for the rapid turnoff of G-protein-coupled receptor signaling pathways (De Vries and Gist Farquhar 1999)
2.13	*CG2727* (*Emp*)	CD36-like	(See *2.7*)
	CG2736	CD36-like	
	CG3829	CD36-like	
	CG3770	Claudin-like	
3.1	*CG6445*[a]	Cadherin-like	Possible role at adherens junctions
	CG3885	Sec3-like exocyst component	Involved in docking at the plasma membrane, which is a function that Lgl has also been implicated in Lehman *et al.* (1999) and Musch *et al.* (2002)
3.2	*CG6190*	Ubiquitin ligase - HECT domain protein	A HECT domain ubiquitin ligase gene related to *hyd*, a tumor suppressor (de Lorenzo *et al.* 1999; Gateff *et al.* 1996)
3.4	*CG5263 (smg)*	Translational repressor	Role in neural cells (Clark *et al.* 2002); possible role in G1-S regulation, given the identification of RpS6 in the screen
1S3	*APC1*[b]	Adenomatous polyposis coli tumor suppressor	Possible role at adherens junction and may function with EB1 (Ahmed *et al.* 1998; Lu *et al.* 2001)
	pins (*rapsinoid*)	Asymmetric division of neuroblasts	Directly interacts with Dlg (Bellaiche *et al.* 2001; Parmentier *et al.* 2000)

(*continued*)

TABLE 7

(Continued)

Suppressor group	Candidate gene	Gene function	Possible links to cell cycle regulation
13S1	*CG5555*	Ring finger domain	Homologous to a protein shown to interact with the BRCA1 tumor suppressor protein in mammals (Sharan and Bradley 1998)
59S9	*Spinophilin* (*neurabin*)	Actin-binding scaffold protein	In mammalian cells is involved in binding to and upregulating Rac and p70-S6K activity (Buchsbaum *et al.* 2003)
59S18	*draper*	Protein with multiple extracellular EGF repeats, similar to laminin γ3 and Notch	Predicted to be involved in cell adhesion and signaling and involved in differentiation of neural cells (Egger *et al.* 2002)
68S10	*canoe*[b]	Component of the Adherens junction	Acts antagonistically to the Ras signaling pathway (Matsuo *et al.* 1997)
	CG12591	Ig C2-domain—cell adhesion	Possible role at adherens junction or in signaling (Tepass *et al.* 2001)

?, failed to complement *2.3* alleles, but testing did not confirm whether the mutations dominantly suppress *DmcycE*^{JP}.
[a] Tested by Southern analysis and no obvious disruptions were observed.
[b] Mutants were tested and shown to complement the *DmcycE*^{JP} suppressor.

the power of Drosophila whole-animal genetics as a tool for revealing new cell proliferation pathways.

It is unclear at present how many of the *DmcycE*^{JP} modifiers identified in our screen bear upon the role of Cyclin E in DNA replication or centrosome duplication (see Introduction). Brahma and Moira are likely to be downstream targets of Cyclin E/cdk2 that may impact upon transcriptional regulation or DNA replication (Brumby *et al.* 2002), but whether other interactors act upstream or downstream of Cyclin E remains to be determined. The only *cyclin E* interactor we identified that has been shown to be associated with the centrosome is EB1 (Rehberg and Graf 2002); however, whether this reflects upon the role for Cyclin E in centrosome duplication in Drosophila is unclear. A recent study has shown that the Drosophila SkpA, a component of SCF ubiquitin ligases, regulates centrosome duplication independently of Cyclin E accumulation (Murphy 2003).

Similar genetic screens carried out using phenotypes generated by overexpression of *cyclin E* (Lane *et al.* 2000) or the G1-S regulators *E2F1/Dp* (Staehling-Hampton *et al.* 1999), *Rbf* (Duman-Scheel *et al.* 2002), and human *p21* (Cdk2 inhibitor; I. Hariharan, personal communication) have revealed a more restricted set of interacting genes than that obtained in our *cyclin E* hypomorphic allele genetic screen. The *GMR-E2F1/Dp* screen (Staehling-Hampton *et al.* 1999) revealed alleles of the chromatin remodeling genes *brm*, *mor*, and *osa* and of the transcription factor *pointed*, an effector of the Egfr-Ras signaling pathway, as enhancers. This is consistent with our identification of *brm* and *mor* as suppressors of the hypomorphic *cyclin E* phenotype in our mutagenesis screen. In addition, we tested alleles of *osa* and showed that they suppressed the hypomorphic *cyclin E* phenotype (Brumby *et al.* 2002). The *sevenless-cyclin E* screen (Lane *et al.* 2000) revealed alleles in identified cell cycle genes *cdk2* (as a suppressor), *dacapo* (as an enhancer), and *E2F1* (a suspected gain-of-function allele as an enhancer) and identified as an enhancer the novel gene *spen* (*poc*), also identified in the *GMR-E2F1/Dp* screen (Staehling-Hampton *et al.* 1999). Spen (Poc) is a RNP-type RNA-binding protein that has recently been shown to be required for Wg signaling in imaginal discs (Lin *et al.* 2003). We have not identified *spen* (*poc*) as a suppressor in our genetic screen, but alleles of *spen* (*poc*) were tested and shown to suppress *DmcycE*^{JP} (Table 2), consistent with the Wg signaling pathway acting to negatively regulate G1-S progression in the eye disc. As detailed above, we have shown that one of the single alleles identified as an enhancer in the *sevenless-cyclin E* screen is allelic to our *DmcycE*^{JP} suppressor *2.11*, which we have identified as *scab*. In the *GMR-Rbf* screen, alleles of *patched*, encoding an inhibitor of Hedgehog (Hh) signaling, were identified as dominant suppressors (Duman-Scheel *et al.* 2002). Although our mutagenesis screen did not reveal alleles of *patched*, *patched* alleles strongly suppressed *DmcycE*^{JP} (Table 2), consistent with the notion that Hh signaling leads to increased transcription of *cyclin E* (Duman-Scheel *et al.* 2002). The greater number of interactors that we obtained in our screen may be due to the fact that our screen was of a *cyclin E* hypomorphic phenotype that affected cell proliferation in early eye development as well as the post-MF S phases and may therefore have been more sensitive to gene dosage than the overexpression screens. Furthermore, unlike the overexpression screens, the *cyclin E* hypomorphic screen is more likely to reveal genes that are upstream of *cyclin E* expression.

The *DmcycE*^{JP} suppressor genes we have identified

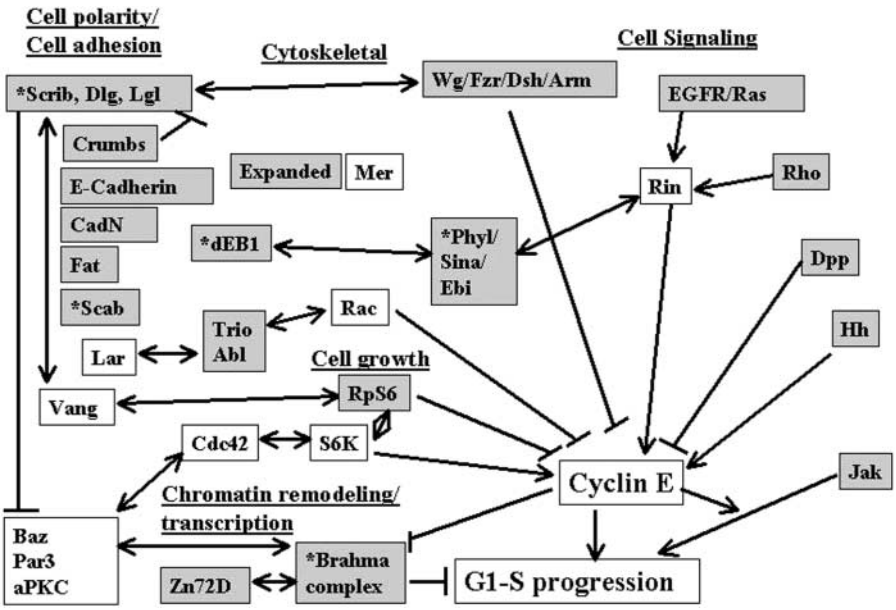

FIGURE 4.—Possible pathways connecting *cyclin E*-interacting genes. Interactors identified in our *cyclin E* screen are shaded. Direct protein interactions between *cyclin E* interactors or other relevant proteins are indicated by the double-headed arrows. Arrows indicate positive interactions while barred lines indicate negative interactions. Not shown are interactions between Scab and the Dpp pathway, between E-cadherin and the Egfr pathway, between Fat and Atrophin (a nuclear corepressor), and between Expanded/Merlin and the Dpp and Egfr pathways. *, genes that genetically interact with *scribble*. See the text for details.

from our mutagenic screen are mostly distinct from Drosophila tumor suppressors previously described (TOROK *et al.* 1993; GATEFF *et al.* 1996; DE LORENZO *et al.* 1999). Recently, clonal screens have revealed a novel pathway involved in inhibiting G1-S progression and cell death in the Drosophila eye (HAY and GUO 2003). This pathway includes *lats* (*warts*), *salvador*, and *hippo*, and although this pathway has been recently shown to regulate *cyclin E* at possibly both a transcriptional and protein stability level, we did not identify alleles of these genes in our genetic screen. Alleles of *hippo*, at least, have been shown to suppress the $DmcycE^{JP}$ phenotype (WU *et al.* 2003). The fact that we did not identify *hippo* in our mutagenesis screen may have been because the screen was not saturating. However, *lats* (*warts*) alleles did not show appreciable suppression of $DmcycE^{JP}$ (Table 2); therefore it is possible that only certain mutations of this pathway are capable of dominant suppression.

Also pertinent to our study is the recent Drosophila protein interaction map determined by yeast two-hybrid analyses (GIOT *et al.* 2003). None of our identified *cyclin E* genetic interactors were identical to the 15 interactors identified by the protein interaction study (GIOT *et al.* 2003), but many proteins identified in our screen were not analyzed in their screen (*e.g.*, Brahma, Moira, Scab, CadN, Dsh, Scribble, Crumbs, Expanded, and Abl). Most of the 15 yeast two-hybrid interactors with Cyclin E are uncharacterized, but of the characterized proteins, Combgap, a transcription factor, has been implicated in cell proliferation via its effect on Ci expression (CAMPBELL and TOMLINSON 2000). Of the other characterized interactors, Gliolectin is involved in cell adhesion in axon pathfinding (SHARROW and TIEMEYER 2001) and Traf2 is involved in Dorsal activation (SHEN *et al.* 2001), but no cell proliferation role has been described for these proteins. Some of the Cyclin E yeast two-hybrid interacting genes map to regions where *cyclin E* genetic interactors have been mapped (not shown) and are candidates for future analysis.

Whether the genetic suppressors of *cyclin E* identified in our screen can all be connected in a common pathway or represent several converging pathways acting upon G1-S progression in the eye imaginal disc remains to be determined. As a first step to explore this we examined interactions between a weak *scrib* mutant and $S(DmcycE^{JP})$ alleles, which revealed genetic interactions with *lgl*, *phyl*, *dEB1 scab*, *mor*, the unidentified suppressors *2.3*, *2.4*, and *2.9*, and to a lesser extent *brm*. This analysis provides a connection between chromatin remodeling, signaling, cytoskeletal, cell-cell adhesion, and cytoarchitectural suppressor genes. How exactly these pathways may be connected and whether other genes identified in the $DmcycE^{JP}$ screen are also functionally connected now warrant further investigation.

Interestingly, many of the genes identified in the screen have roles in cell polarity; for example, *scrib*, *dlg*, *lgl*, and *crumbs* are involved in apical-basal cell polarity, while *dlg*, *fat*, *expanded*, and the Wg pathway, via Rho and Jnk, have roles in planar polarity (BLAUMUELLER and MLODZIK 2000; BELLAICHE *et al.* 2001; YANG *et al.* 2002; EATON 2003; FANTO *et al.* 2003). Moreover, *E-cadherin* (*shg*) and β-*catenin* (*arm*) function at the adherens junction, which is important in both apical-basal cell polarity and cell-cell adhesion (TEPASS *et al.* 2001). Whether other cell polarity genes, such as *bazooka*, *par3*, *apkc*, *patj*, and *stardust* (HUMBERT *et al.* 2003), are also $DmcycE^{JP}$ modifiers and the molecular mechanism by which this occurs require further analysis. Pertinent to this, a recent study has shown that *apkc* clones have reduced cell division and that *apkc* mutants can suppress the overgrowth of *lgl* mutants, suggesting that upregulation of *apkc* contributes to the overgrowth phenotype of *lgl*, and perhaps also *scrib* and *dlg*, mutants (ROLLS *et al.* 2003).

How are junctional components connected to signal-

ing pathways or to the cell cycle machinery? In mammalian cells, the Frizzled receptors, Fz1, Fz2, Fz4, and Fz7, have been shown to bind to mammalian Dlg1 (Hering and Sheng 2002), which may therefore provide a connection between apical-basal and the Frizzled-Rho-Jnk planar polarity pathway (Adler and Lee 2001), as well as to the canonical Wg-Arm (β-catenin) pathway to effect S-phase entry (Figure 4). Furthermore, mammalian *scrib* genetically and physically interacts with the planar polarity gene, *vang* (*strabismus*) (Kallay *et al.* 2003; Montcouquiol *et al.* 2003; Murdoch *et al.* 2003). Mammalian Vang is a potential tumor suppressor that can act to regulate the Wg-Arm pathway (Katoh 2002). If Vang acts similarly in Drosophila, it would provide another connection between planar polarity, apical-basal polarity, and Wg signaling pathways. Connections between polarity proteins and the Egfr signaling pathway have also been observed in *Caenorhabditis elegans* and mammalian cells (Simske *et al.* 1996; Huang *et al.* 2003). Consistent with this, antagonistic interactions between E-cadherin in adherens junction function and the Egfr signaling pathway have been observed in the Drosophila nervous system (Dumstrei *et al.* 2002), and if this also occurs in the eye imaginal disc then decreasing E-cadherin levels would be expected to cause an increase in Egfr-Ras signaling that would lead to increased Cyclin E protein (Brumby and Richardson 2003). Furthermore, there is evidence that the FERM domain protein Expanded, which functions together with another FERM domain protein, Merlin, a homolog of the NF2 tumor suppressor, modulates the Dpp signaling pathway (McCartney *et al.* 2000) and in mammalian cells NF2 can inhibit Ras signaling (Lim *et al.* 2003). There is also a precedent for a connection between Integrin signaling and cell polarity pathways, since the transmembrane Laminin receptor Dystroglycan has been shown to have a role in epithelial cell apical-basal polarity (Deng *et al.* 2003). This now raises the question of whether Scab (αPS3-Integrin) plays a role in apical-basal cell polarity. In mammalian cells, integrins act via focal adhesion kinase (Fak) to activate Rho-family GTPases (Schoenwaelder and Burridge 1999) and recently it has been reported that integrins are important for the localization of aPKC (Datta *et al.* 2003). In Drosophila, a role for *scab* in modulation of Dpp signaling has been described in wing vein formation (Araujo *et al.* 2003), suggesting a mechanism by which *scab* may also affect cell proliferation. Furthermore, there is a connection between the Trio-Rac-Abl pathway and polarity, since Trio interacts with the Lar receptor-like tyrosine phosphatase, which has recently been shown to have a role in epithelial planar polarity (Frydman and Spradling 2001).

The Drosophila microtubule-binding protein dEB1 has also been implicated in playing a role in adherens junction function and cell polarity by RNA ablation studies (Lu *et al.* 2001; Rogers *et al.* 2002). Interestingly, the recently published study on Drosophila protein interactions using yeast two-hybrid analysis has revealed that dEB1 binds to the Sina homolog *CG13030*, providing a connection to the Sina-Phyl pathway (Giot *et al.* 2003). Sina and the Sina homolog also bind to Rasputin (Rin), a homolog of the RasGAP-binding protein G3BP, which has a role in planar polarity via effects on the Rho signaling pathway (Pazman *et al.* 2000). Thus the Sina-Phyl complex may act via Rasputin to negatively regulate Ras and Rho signaling and thereby G1-S progression (Figure 4). The protein interaction study (Giot *et al.* 2003) has also revealed that RpS6, identified as a suppressor in our screen, binds to the planar polarity protein Vang/Strabismus, which was not tested in our screen. Interestingly in mammalian cells, Cdc42, a Rho-family GTPase component of the apical Par6 complex, functions via p70-S6 kinase to upregulate *cyclin E* transcription (Chou *et al.* 2003) and disruption of RpS6 in mice results in a specific block in *cyclin E* expression (Volarevic *et al.* 2000). The yeast two-hybrid analysis study (Giot *et al.* 2003) also revealed protein interactions between Zn72D and Actin 5C, a component of the Brahma complex (Papoulas *et al.* 1998), between the Brahma-associated protein Bap60 and the apical zone polarity protein aPKC (Humbert *et al.* 2003), and between Dlg or Lgl and zinc finger transcription factors. There are precedents for functional interactions between cell polarity proteins and nuclear corepressors, for example, between Drosophila Fat (atypical cadherin involved in planar polarity) and Atrophin (Fanto *et al.* 2003), suggesting that yeast two-hybrid interactions between the Brahma complex or the zinc finger transcription factors and cell polarity proteins may be functionally relevant, although further investigation is required. Although there may be many pathways that connect the Cyclin E interactors identified in this screen to G1-S progression, the examples above suggest ways in which cell polarity proteins may link to signaling pathways or directly to chromatin remodeling, corepressors, or transcription factors to regulate *cyclin E* or the transcription of other G1- to S-phase genes (Figure 4).

In summary, the identification in our *cyclin E* screen of genes that were not necessarily predicted to play roles in G1-S progression highlights the importance of using whole-animal genetics to investigate G1-S regulation. The identified *cyclin E* genetic suppressors are conserved in mammals and given their demonstrated or presumptive roles as inhibitors of G1-S progression in Drosophila are candidates for tumor suppressors in mammalian cancers.

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