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DNA replication machinery is required for development in Drosophila

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1. ABSTRACT

In *Drosophila*, some factors involved in chromosome replication seem to be involved in gene amplification and endoreplication, which are actively utilized in particular tissue development, but direct evidence has not been shown. Therefore, we examined the effect of depletion of replication factors on these processes. First, we confirmed RNAi knockdown can be used for the depletion of replication factors by comparing the phenotypes of RNAi knockdown and deletion or point mutants of the components of DNA licensing factor, MCM2, MCM4 and Cdt1. Next, we found that tissue-specific RNAi knockdown of replication factors caused tissue-specific defects, probably due to defects in DNA replication. In particular, we found that depletion inhibited gene amplification of the chorion gene in follicle cells and endoreplication in salivary glands, showing that chromosomal DNA replication factors are required for these processes. Finally, using RNAi, we screened the genes for chromosomal DNA replication that affected tissue development. Interestingly, wing specific knockdown of Mcm10 induced wing formation defects. These results suggest that some components of chromosomal replication machinery are directly involved in tissue development.

2. INTRODUCTION

DNA replication machinery is essential for growth of all cells. Origin recognition complex is the platform of DNA replication initiation complex. Cdt1-Mcm2-7 is thought to be the licensing factor

which solely directs the S phase per one cell cycle. A lot of evidence indicates that Mcm2-7 is also a replicative helicase (1). Recently, Botchan *et al*. showed that a complex including CDC45, Mcm2-7, and GINS (2) (CMG complex)(3) is the eukaryotic DNA replication fork complex in *Drosophila* embryo. DNA polymerases, including Polα-primase, Polδ and Polε, are the enzymes responsible for the elongation phase of DNA replication. The yeast CDC6 is a loader of Mcm2-7 onto DNA replication origin (4). However, higher eukaryotic CDC6 function has not been clarified.

Over many years, a lot of mutants become isolated. Among them, homozygotes are lethal during their development. Their tissue specificity is not known. In Drosophila, a tissue-specific RNAi knockdown system is available in combination with a Gal4-UAS system (5). The transgene of interest, which is expressed with a Gal4-dependent promoter, is introduced into the fly embryo. By crossing with a fly expressing Gal4 in a tissue-specific manner (Gal4 driver), one can obtain the flies that express the transgene in a tissue-specific manner. When the anti-sense RNA against the target gene is expressed using the tissue-specific Gal4 driver system, the RNA transcribed from the target DNA forms dsRNA, which could be destroyed by RNAi machinery, resulting in the depletion of the target gene product.

We have studied DNA replication and chromatin structure using polyoma virus (6,7) and the budding yeast *Saccharomyces serevisiae* (8,9). In particular, in *Drosophila* tissues, chromosomal abnormalities, including gene amplification and endoreplication, occur in a developmentally regulated manner (10,11,12). However, it is not known if the DNA replication machinery required for chromosome replication in the mitotic cell cycle is needed for gene amplification and endoreplication.

In this study, we performed knockdown of chromosomal DNA replication licensing factor complex subunits Mcm2, Mcm4, Cdt1, and CDC6. First, we showed that the RNAi knockdown of chromosomal DNA replication licensing factor complex subunits Mcm2, Mcm4, and Cdt1 could induce the same phenotype as that of the mutants that these genes exhibit. Next, we tried to knock down several genes seemingly involved in gene amplification and endoreplication by the RNAi system combined with Gal4 driver in *Drosophila*. We demonstrated that knockdown of DNA replication resulted in tissue-specific defects, probably through defects in DNA replication. These findings showed that chromosomal DNA replication machinery plays an active role in tissue development in *Drosophila*. Finally, using the RNAi system, we screened the DNA replication machinery genes.

3. MATRERIALS AND METHODS

3.1. Fly stocks

Fly stocks were maintained under standard conditions. The RNAi knockdown lines were obtained from the National Institute of Genetics (Mishima, Japan) and Vienna Drosophila RNAi Center (Vienna, Austria). C323a-Gal4 (yw), sp/CyOGFP (yw), pre/TM6BGFP (yw), Gla/CyoGFP, E2Fi1, Dpa1, lat1, dpa1, rL074, dup, and geminin were received from the Bloomington Drosophila Stock Center (Indiana, USA). *Eyeless-Gal4/Cyo, Actin 5C/TM3(GFP), tubulin-Gal4/TM6B(GFP), Vg-Gal4, en-Gal4, and Sg-Gal4/FM7* were gifts from Dr. Masamitsu Yamaguchi. Orc2 mutant k43, E2F1 91, UAS-Orc1, UAS-Orc2, and UAS-CDC6 were received from Dr. Maki Asano. Other lines are listed in Table 1. All RNAi flies were received from the Vienna Drosophila RNAi Center (VDRC).

3.2. Quantitative reverse transcriptase-PCR

Total RNA was isolated using Trizol Reagent (Invitrogen). Oligi dT primers and a Takara high fidelity RNA PCT kit (Takara, Kyoto, Japan) were used for generation of complementary DNA. Thereafter, real-time PCR was performed using a SYBR Green I kit (Takara) and the Applied Biocystems 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). RNA expression efficiencies decreased to 25% in every case.

3.3. Scanning electron microscope

The eyes of the mutant flies were observed with a VE-7800 scanning electron microscope (Keyence, Osaka, Japan) in the high vacuum mode.

4. RESULTS

4.1. Knockdown of DNA replication machinery by Act5C- and tubulin-Gal4 drivers was lethal during Drosophila development

The tissue-specific knockdown of gene expression in RNAi knockdown flies resembled the Cre-loxP system in mice. In *Drosophila*, various flies temporally expressing Gal4 in various specific tissues (Gal4 driver) have been stocked. A fly having UAS-shRNA interference transgene construct crosses to the Gal4 driver. The mRNA of the target gene of UAS-shRNA was eliminated temporally and spatially in a tissue-specific manner. The fly Figure was downloaded from Flybase, which is a database of *Drosophila* DNA, chromosomes, proteins, fly mutants, and stocks.

In the initial strategy for researching RNAi availability in *Drosophila*, we aimed to compare the phenotypes of the flies in which the particular genes were knocked down in the embryonic stage and

Table 1. List of Drosophila mutants

Mutants	Source
k43	Bloomington Center
Lat1	Bloomington Center
SH0065	Szeged Center
rL074	Center
Dpa1	Center
SH0520	Szeged Center
Dupa1	Bloomington Center
Dupa3	Bloomington Center
Dup enhancer trap	Bloomington Center
GemininK09107	Bloomington Center
GemininK1409	Bloomington Center
Psf1 enhancer trap	Bloomington Center
SH0805(Psf1 enhancer trap)	Szeged Center
Cdc7 enhancer trap	Bloomington Center
CycE01672	M. Lilly, NIH
MH30	J.S. Lipsick, Stanford University
MH107	J.S. Lipsick, Stanford University
E2F1 91	Bloomington Center
E2Fi2	Bloomington Center
DPa1	Bloomington Center
Cyclin A	Bloomington Center
$Cdc20^{FZY}$	Bloomington Center
$Cdh1^{FZR}$	Bloomington Center
Rca1/Emi1	Bloomington Center
Cyclin A & Rca1/Emi1	Bloomington Center
UAS-ORC1-GFP	M. Asano, Duke University Medical Center
UAS-ORC2-GFP	M. Asano, Duke University Medical Center
UAS-CDC6	M. Asano, Duke University Medical Center
UAS-GFP-GFP	M. Asano, Duke University Medical Center

whole body and their mutants. First, we performed knockdown of DNA replication machinery by *Act5C*- and *tubulin*-Gal4 drivers, which express target genes in whole body. The knockdown of Mcm2, Mcm4, Cdt1, and Cdc6 resulted in lethality (Tables 2, 3). We compared the lethal phases of Act5C- and tubulin-Gal4 drivers (Table. 4). The data showed almost the same phenotype. Next, we searched the lethal phases of null mutants. The knockdown of these genes led to lethality before the 3rd larvae stage and the flies never grew to adults. However, their homologous mutants (Table 5), rL074 (Mcm2 enhancer trap line (13); β-galactosidase was inserted in mcm2 promoter), dpa1 (transcription factor DP mutant), dupa3 (Drosophila cdt1 mutant), and dup enhancer trap line (dup enhancer trap line; β-galactosidase was inserted in cdt1 promoter) showed the same phenotype as the knockdown flies of these genes. These findings showed that RNAi knockdown methods have an equal effect on mutants.

4.2. The knockdown of DNA replication machinery by *eyeless-Gal4* driver resulted in abnormal eye-antenna structure

We tried to knock down components of DNA replication machinery in tissues that undergo mitotic cell cycles. We choose the eyeless-Gal4 driver, which mainly expresses target genes in the eye-antennal primordia and central nervous system in the eye imaginal disc of the late embryo that is in the growing stage. As shown in Figure 1, knockdown of any of Mcm2, Mcm4, Cdc6, Cdc45, Psf2, Polα, Polε and Polδ caused a small-eye phenotype (Figure 1 and Table 6). In severe cases, eye and antenna were completely lost. In the case of

Table 2. Lethal phase of knockdown experiments by Gal4 driver *Act5C-Gal4*

Responder (UAS-IR)	Chromosome linkage	Phenotype (adult)	Adult escaper /total flies (%)
Mcm2	III	Lethal	0 (N=194)
Mcm3	III	Lethal	0 (N=7)
Mcm4IR-1	II	Lethal	0.5.4 (N=185)
Mcm4IR-2	III	Lethal	0.3.3 (N=305)
Mcm5IR-1	III	Lethal	0 (N=340)
Mcm5IR-2	III	Lethal	0 (N=131)
Cdc6IR-3	II	Lethal	0.5.3 (N=375)
Cdc6IR-1	III	Lethal	0.8.8 (N=114)
Cdt1IR-1	II	Lethal	0.8.7 (N=230)
Cdt1IR-2	III	Lethal	0.4.9 (N=406)
Orc4IR-3	II	Lethal	2.0.0 (N=250)
Orc4IR-2	III	Lethal	7.8.5 (N=395)
Orc5IR-1	II	Lethal	0.5.7 (N=350)
Orc5IR-2	III	Lethal	0.5.5 (N=182)
Orc6	II	Severe growth defect*	13.0.7 (N=176)
Cdc45IR-1	II	Lethal	0.3.0 (N=334
Cdc45IR-2	III	Lethal	2.9.2 (N=137)
RPA70IR-1	II	Lethal	1.2.3 (N=316)
RPA70IR-2	III	Lethal	0.3.2 (N=317)
Psf1 (VDRC)	III	Lethal	0 (N=163)
Psf1 IR-3	III	Lethal	0.8.9 (N=225)
Psf2 IR-4	II	Lethal	0 (N=116)
Psf2 IR-1	III	Lethal	1.9.4 (N=155)
Rfc3 IR-1	II	Lethal	1.5.6 (N=321)
Rfc3 IR-9	II	Lethal	1.8.2 (N=329)
Mcm10	III	Lethal	7.8.7 (N=127)
Polα180IR-1	III	Lethal	0 (N=125)
Polα180IR-3	III	Lethal	0 (N=169)
Polα50IR-1	II	Lethal	0 (N=19)
Polα50IR-2	III	Lethal	0 (N=262)
Polα73IR-1	II	Lethal	0 (N=106)
Polα73IR-2	II	Lethal	0 (N=73)
Polδ IR-1	II	Lethal	6.1.8 (N=372)
Polδ IR-2	III	Lethal	0 (N=20)
Polε255IR-1	II	Lethal	0 (N=196)
Polε255IR-2	III	Lethal	2.9.6 (N=109)
DP IR-1	II	Lethal	0 (N=109)
DP IR-2	III	Lethal	5.1.5 (N=136)
Cul-4	II	Lethal	0 (N=78)
Cul-1	II	Severe growth defect*	14.3.9 (N=132)
dskip-1/SkpA	III	Lethal	0.6.5 (N=153)
Rbx1/Roc1	III	Lethal	1.5.4 (N=130)
Roc1b/Roc2	III	Lethal	0 (N=149)
Elongin C	III	Severe growth defect*	14.1.2 (N=255)
yw		Normal	(N=270)

Act5C-Gal4 is expressed in the whole body from early phase.
*Severe growth defect: below 20% of adult escapers

Table 3. Lethal phase of knockdown experiments by Gal4 driver *tubulin-p-Gal4*

Responder (UAS-IR)	Chromosome linkage	Phenotype (adult)	Adult escaper/total flies (%)
Mcm2	III	Lethal	0 (N=133)
Mcm3	III	Lethal	0 (N=154)
Mcm4IR-1	II	Lethal	1.6.4 (N=427)
Mcm4IR-2	III	Lethal	2.0.9 (N=335)
Mcm5IR-1	III	Lethal	0 (N=280)
Mcm5IR-2	III	Lethal	0 (N=106)
Cdc6IR-3	II	Lethal	0 (N=194)
Cdc6IR-1	III	Lethal	0 (N=52)
Cdt1IR-1	II	Lethal	4.6.1 (N=347)
Cdt1IR-2	III	Lethal	0 (N=152)
Orc4IR-3	II	Lethal	0 (N=137)
Orc4IR-2	III	Lethal	0.8.3 (N=360)
Orc5IR-1	II	Lethal	0 (N=179)
Orc5IR-2	III	Lethal	2.2.6 (N=265)
Orc6	II	Lethal	2.5.6 (N=265)
Cdc45IR-1	II	Lethal	0 (N=39)
Cdc45IR-2	III	Lethal	0 (N=57)
RPA70IR-1	II	Lethal	0 (N=75)
RPA70IR-2	III	Lethal	0 (N=105)
Psf1 IR-1	II	Lethal	0 (N=173)
Psf1 IR-2	III	Lethal	0 (N=137)
Psf2 IR-4	II	Lethal	0.0.9 (N=231)
Psf2 IR-1	III	Lethal	1.6.1 (N=186)
Rfc3 IR-1	II	Lethal	0 (N=178)
Rfc3 IR-9	II	Lethal	0 (N=129)
Mcm10	III	Almost Normal*	64.2.9 (N=340)
Polα180IR-1	III	Lethal	2.5.4 (N=276)
Polα180IR-3	III	Lethal	7.0.8 (N=113)
Polα50IR-1	II	Lethal	1.3.6 (N=220)
Polα50IR-2	III	Severe growth defect**	18.0.2 (N=250)
Polα73IR-1	II	Lethal	0 (N=105)
Polα73IR-2	II	Lethal	0 (N=150)
Polδ125KDa IR-1	II	Lethal	0 (N=215)
Polδ125KDa IR-2	III	Lethal	0 (N=126)
Polε255IR-1	II	Lethal	1.1.8 (N=254)
Polε255IR-2	III	Slight growth defect	31.4.8 (N=162)
DP IR-1	II	Lethal	2.0.4 (N=98)
DP IR-2	III	Lethal	4.7.9 (N=146)
Cul-4	II	Lethal	0 (N=78)
Cul-1	II	Severe growth defect**	14.3.9 (N=132)
dskip-1/SkpA	III	Lethal	0 (N=20)
Rbx1/Roc1	III	Lethal	1.5.4 (N=130)
Roc1b/Roc2	III	Lethal	0 (N=132)
Elongin C	III	Severe growth defect**	12.1.2 (N=132)
yw		Normal	(N=198)

Tubulin-p-Gal4 is expressed in the whole body from early phase. * Almost Normal: above 60% of adult escapers ** Severe growth defect: below 20% of adult escapers *** Slight growth defect: about 30% of adult escapers

Table 4. Lethal phase of *Actin5C-Gal4* or *tubulin-p-Gal4>>IR*

Responder (UAS-IR)	Act5C-Gal4	Tubulin-p-Gal4
Orc5	L-P	-L
Mcm2	L	L
Mcm4	-L	-L
Mcm5	L-P	L-P
CDC6	P-A	L
Cdt1	-L	- L

Stage of lethal phase shows P, L, and A. P: papae, L: larvae, A: adult

Table 5. Lethal phase of null mutants

Gene	Mutant	Chromosome linkage	Stage of lethality
Orc2	k43	III	P-A
Orc3	Lat1	II	L-P
Orc3	SH0065	II	L-P
Mcm2	rL074	III	P-A
Mcm4	Dpa1	II	L-P
Mcm4	SH0520	II	L-P
Cdt1	Dupa1	II	P
Cdt1	Dupa3	II	L
Cdt1	Dup enhancer trap	II	L
Geminin	GeminiK09107	II	L
Geminin	GeminiK1409	II	L
Psf1	Psf1 enhancer trap	II	P
Psf2	SH0805(Psf1 enhancer trap)	II	- L
Cdc7	Cdc7 enhancer trap	X	P-A
CycE	CycE01672	II	P-A
Myb	MH30	X	L-P
Myb	MH107	X	L
E2F1	E2F1 91	III	L
E2F1	E2Fi2	III	L-P
DP	DPa1	II	- L
Cyclin A	Cyc A	III	Embryonic lethal
$Cdc20^{FZY}$	fzy^{4}	II	Embryonic lethal
$Cdh1^{FZR}$	Rap	X	Embryonic lethal
Rca1/Emi1	$Rca1^{1}$	II	Embryonic lethal
Cyclin A & Rca1/Emi1			Embryonic lethal

Stage of lethal phase shows P, L, and A. P: papae, L: larvae, A: adult

Cdt1, we could not find adults having an eyeless-Gal4 or Cdt1 RNAi construct. These findings suggest that the knockdown of Cdt1 led to lethality, probably because of a headless phenotype. Morphogenesis in multicellular organisms is associated with patterned cell proliferation. As a rule, the size of an organ and its shape are specific. Knockdown of DNA replication machinery terminated the fate; therefore, the specificity disappeared.

4.3. Small-eye phenotype of Cdc6 IR rescued by overexpressed Orc1 and Orc2

Cdc6 and Orc1 have high similarity of amino acid sequences. In *S. pombe*, overproduction of Cdc6 homologue and Cdc18 induces re-replication. Thus, we investigated whether Cdc6IR is rescued by overexpression of Orc1 and Orc2 (Figure 2 and Table 7).

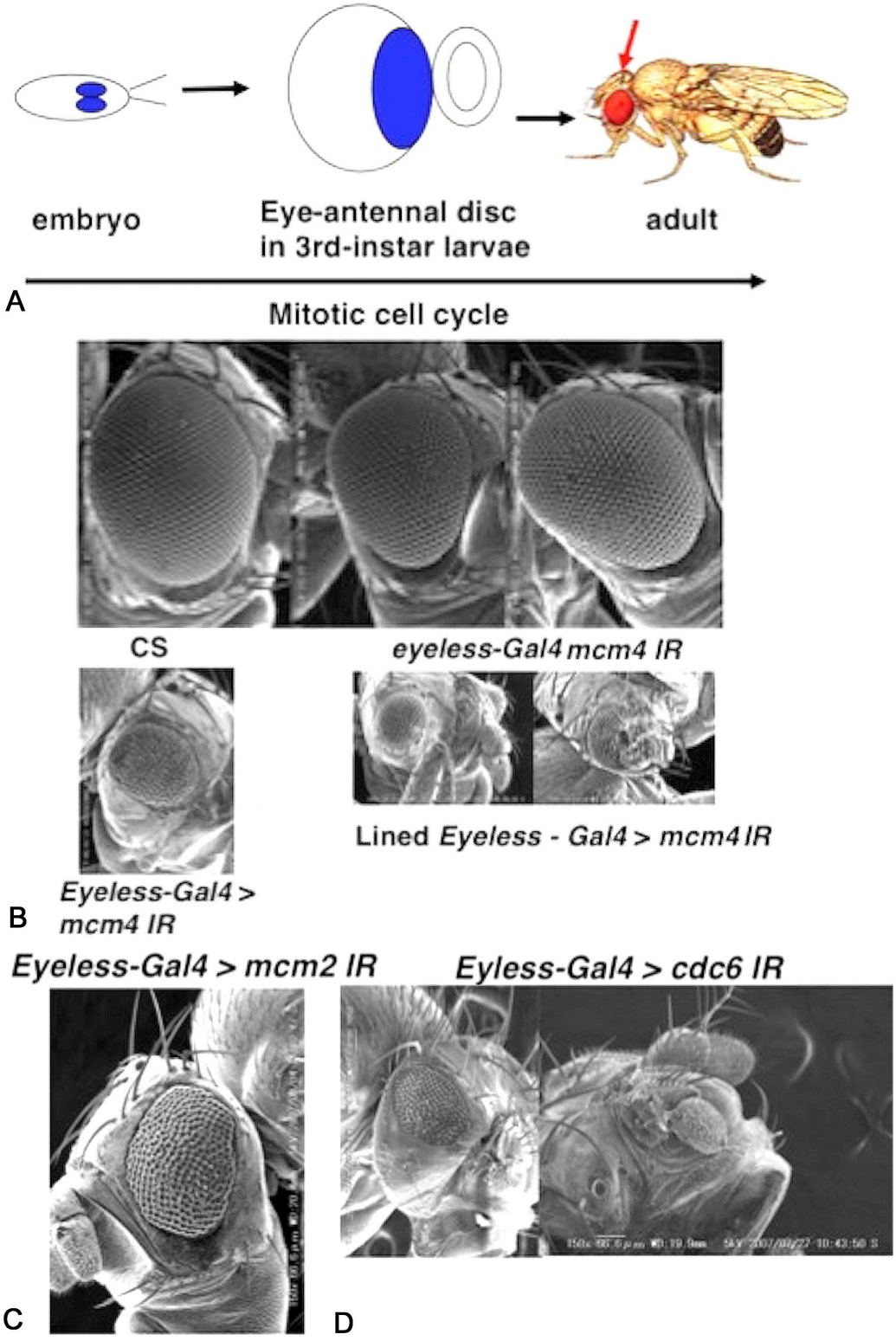

Figure 1. Knockdown in eye-antenna development, including mitotic cell cycle. A. Eyeless-Gal4 is expressed in eye-antennal primordia in late embryo. In third-instar larvae, eyeless-Gal4 is expressed in the anterior of the eye disc. As a result, the eye structure changed. B. Small eyes were found by the knockdown of Mcm4, which is a subunit of MCM complex, a licensing factor/putative replicative helicase complex. CS: Carton S; *eyeless-Gal4*: e*yeless-Gal4, mcm4-IR (UAS-mcm4)*; ">": two lines were crossed. "Lined" shows the flies have *eyeless-Gal4* and *UAS-mcm4* every generation. C. The knockdown of Mcm2, which is a subunit of MCM complex. D. The knockdown of CDC6, which is an essential factor for the formation of pre-replicative complex on the replication origins. E. RNAi-dependent knockdown of DNA replication machinery by eyeless-Gal4 driver: target proteins were CDC45, Psf2 (which is a subunit of GINS), Polα-primase 180KDa, 73KDa subunit, Polε 255KDa subunit, Polδ 255KDa subunit and Rfc3(one subunit RFC).

Table 6. Lethal phase of knockdown experiments by Gal4 driver *eyeless-Gal4*

Responder (UAS-IR)	Chromosome linkage	Phenotype (adult)
Mcm2	III	Small eye (49.2.6%; N = 136)
Mcm4	II	Small eye (100%; N= 90)
CDC6	II	Small eye (53.7.7%; N= 106)
Cdt1	II	Headless (**0%; N = 130)**
CDC45	II	Small eye (75.0.0%; N = 48)
Psf2	II	Small eye (18.0.3%; N= 124)
Rfc3	II	Small eye (31.7.5%; N = 126)
Mcm10	II	Small eye (67.6.1%; N = 71)
Polα 180KDa	III	Small eye (6.2.5%; N=112)
Polα 73KDa	II	Small eye (6.2.5%; N=112)
Polδ 125KDa	II	Small eye (33.3.3%; N = 126)
Polε255KDaIR-1	II	Small eye (38.6.1%; N = 313)
Polε255KDaIR-2	III	Small eye (25.7.6%; N = 198)

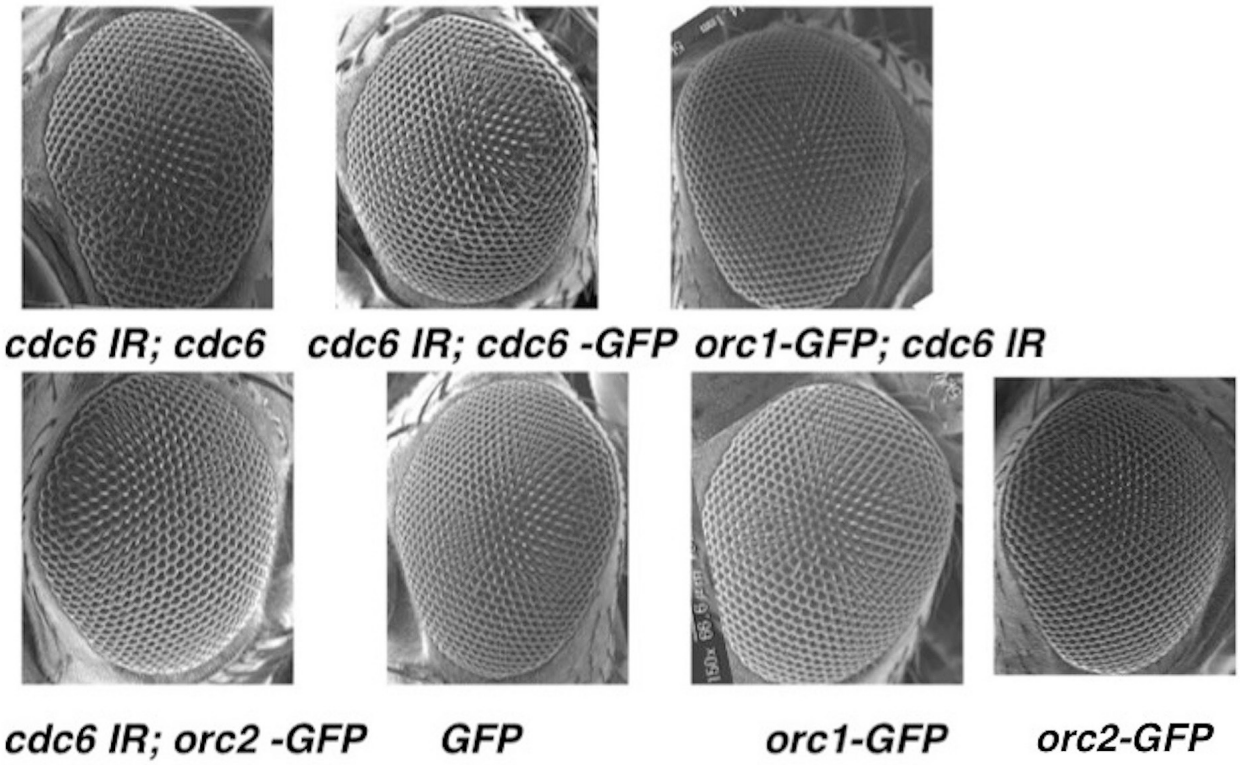

Figure 2. Small-eye phenotype of Cdc6 IR rescued by overexpressed Orc1, Orc2–GFP, and UAS-GFP.

4.4. The knockdown of DNA replication machinery by *c323-Gal4* driver in follicle cells was sterile

Each cell or tissue has its specific cell cycle in *Drosophila* (10,11). In follicle cells, endoreplication and gene amplification occur to amplify the genes encoding egg shell protein for eggshell formation (10,11,14). We performed knockdown of DNA replication machinery by *c323a-Gal4* driver, which expresses target gene products in follicle cells from stage 7, at which stage endoreplication and gene amplification occur. As shown in Figure 8, knockdown of replication proteins, Mcm2, Mcm4, and Cdc6, caused a sterile phenotype or growth defect. These findings suggest that endoreplication and gene amplification in follicle cells were suppressed, resulting

Table 7. Phenotype of overexpression by *c323a-Gal4*

Responder (UAS)	Chromosome linkage	Phenotype (adult)
ORC1GFP*	II	Female sterile
ORC1GFP*	III	Female sterile
ORC2GFP*	III	Female sterile
CDC6GFP**	II	Normal
CDC6GFP**	III	Normal

* Indicated heterozygous cdc6-GFP. ** Showed homozygous cdc6-GFP.

Table 8. Lethal phase of knockdown experiments by Gal4 driver *c323a*

Responder (UAS-IR)	Chromosome linkage	Phenotype (adult)
Mcm4	II	Female sterile
CDC6	II	Female sterile
CDC45	II	Female sterile
Psf2	II	Female sterile
Rfc3	II	Female sterile
Polα 180KDa	III	Female sterile
Polα 73KDa	II	Female sterile
Polδ	II	Female sterile

Table 9. Summary of phenotypes induced by knockdown of Mcm10 with each Gal4 driver

Gal4 driver	Phenotype
Act5C	Lethal
Tubulin-p-Gal4	Wing phenotype
SD	Wing phenotype
Vg	Normal
en	Normal
Eyeless	Normal

in a defect in oogenesis (15). On the other hand, Orc1, Orc2, and Cdc6 were overexpressed by *c323a*. Cdc6 overexpression showed normal development, but Orc1 and Orc2 cases showed female sterility.

4.5. E2F1-DP transcription factor is involved in development

E2F1-DP heterodimer is a key regulator of cell cycle progression (16,17,18). In *Drosophila,* the factors have another function, chorion gene amplification (19,20). We checked this function in development. Null mutants showed lethality up to larvae (Table 5). We then performed the knockdown of DP by Act5c and tubulin-p-Gal4. DP knockdown with act5C (act5c>>DP IR) or tublin-p-Gal4 (tublin-p-Gal4>>DP IR) showed lethal mutagenesis in flies. As DP needs transcription factor E2F1 function (16), these findings showed that E2F1-DP is involved in development (Tables 2, 3, and 5).

4.6. Null mutants of protein degradation are maybe involved in development

Protein degradation is a key step in the cell cycle. Cul-4, Cul-1, and Rbx1 are components of SCF complex (21). Cyclin A, Cdc20FZY, Cdh1FZR, and Rca1/Emi1 are components of APC (21,22,23,24). We showed *Drosophila* Orc complex was degraded by APC (25). We studied the effects of these proteins in development (Table 5). We then performed the knockdown of components of SCF complex by A*ct5c* and *tubulin-p-Gal4*. We found these flies have lethality (Tables 2, 3). These findings showed that SCF complex is involved in development (Tables 2, 3, and 5).

4.7. Mcm10 is maybe involved in wing formation

We hypothesized that DNA replication machinery contributes to development directly. First,

Table 10. Phenotype of *SD-Gal4 > IR*

Responder (UAS-IR)	Chromosome linkage	Phenotype (adult)
Mcm3	III	Normal
Mcm10	III	Wing phenotype
Polα 50KDaIR-2	III	Wing phenotype
Polε255KDaIR-1	II	Normal

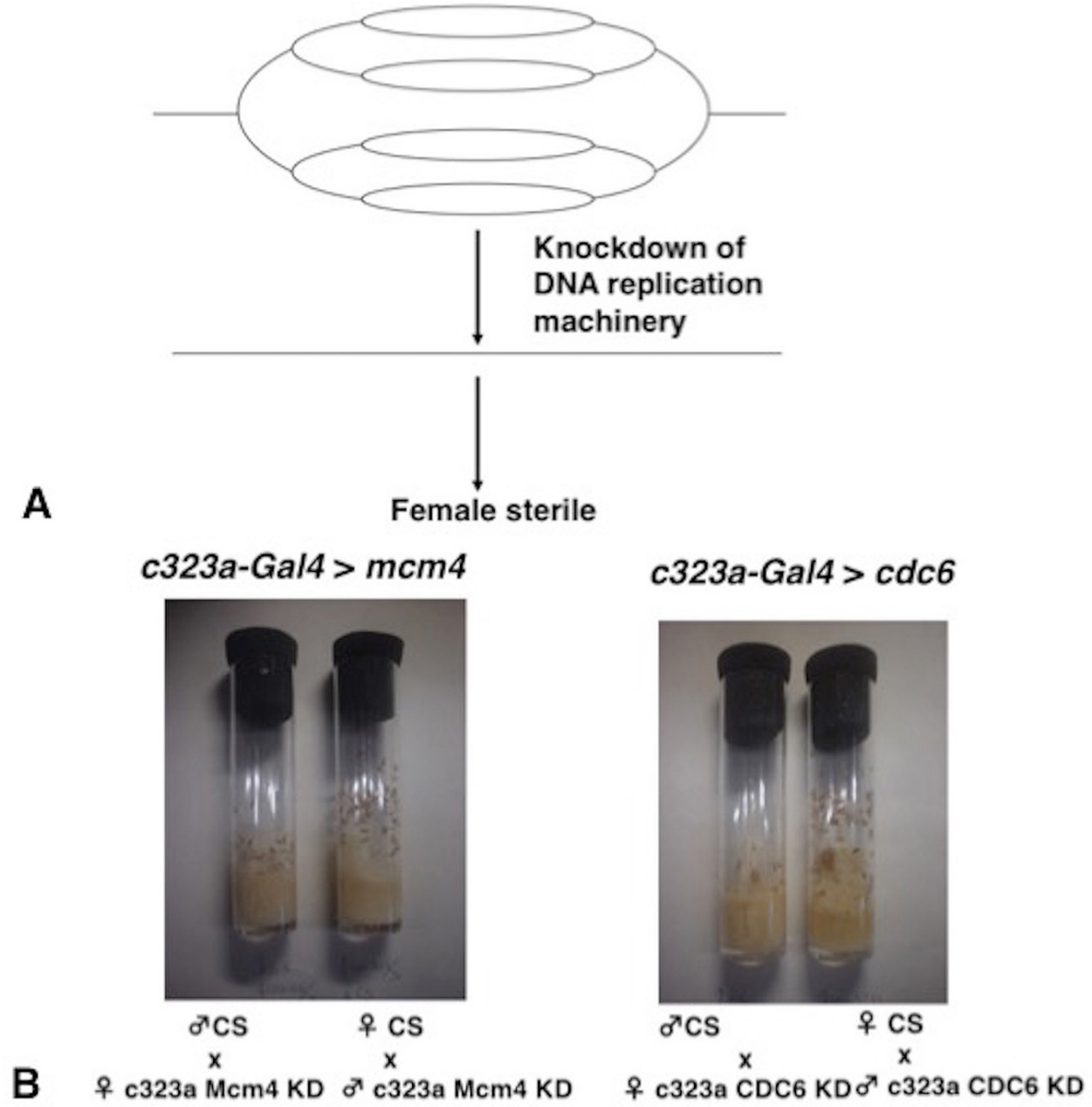

Figure 3. Gene amplification of *Drosophila* follicle cell. A. The amplified region in follicle cells has a so-called onionskin structure. This region, which codes proteins necessary for egg formation and development. B. RNAi knockdown of DNA replication machinery by c323a Gal4 driver.

we performed the knockdown of DNA replication machinery by several Gal4 drivers for screening (Table 9). Surprisingly, knockdown of Mcm10 by *tubulin-p-* and *SD-Gal4* led to wing formation defects. The knockdown of Mcm10 by wing-specific Gal4 and *SD-Gal4* showed wing defects, but not by *Vg* or e*n-Gal4* driver. We performed the knockdown of several DNA replication machineries, including Mcm3 and Polε255KDa, by *SD-Gal4,* but we could not find a wing phenotype (Table 10). These findings suggest that Mcm10 has another function in addition to its function in DNA replication.

5. DISCUSSION

5.1.The knockdown of DNA replication machinery by several Gal4 drivers disturbed mitotic cell cycle, endoreplication, and gene amplification

In this study, to show the function of the replication machineries during development *in vivo*, we performed systematic knockdown of the genes involved in DNA replication machinery and found that many of the gene knockdowns abolished several *Drosophila* tissue organizations. We investigated the relationship between transcription factors and DNA replication origin selection (27). During development, origin seems to be selected strictly (10,11). As transcription factors are terminal targets of signal transduction, we proposed these factors may contribute the selection (28). Therefore, well-controlled DNA replication is an essential part of precise development in many tissues (28,29,30).

In tissues of several insects, including *Drosophila,* and plants, chromosomal abnormalities, including gene amplification and endoreplication, occur in a developmentally regulated manner (31,32) (Figure 3A). However, it is not known if DNA replication machinery is needed for these developmental stages. As shown in Table 3B, c323a Gal4 or CS cross mcm4 and cdc6. *c323a-Gal4 > mcm4* and *c323a-Gal4 > cdc6* bore less eggs than CS. These data show that all knockdown lines of DNA replication factors tested were female sterile (Table 8). *C323a-Gal4* expresses follicle cells and it is known that gene amplification is essential in follicle cells. This suggests that DNA replication machinery is needed for not only mitotic cell cycle but also gene amplification. Though one can imagine the inhibition of DNA replication components can easily affect development of most organs, our exhaustive findings provide evidence that support this hypothesis.

Chromosomal abnormalities, including translocation, inversion, gene amplification, and endoreplication, induce polyploidy or aneuploidy, which is associated with almost all cancer cells. Many kinds of leukemic cells have chromosomal translocation and express resulting oncogenic chimeric proteins. For example, in acute myeloblastic leukemia (AML) M2 phase, t (8; 21) chromosomal translocation is found in many cases, and as a result, oncogenic Runx1 (AML1)-ETO (MTG8) is expressed (33). In clinical trials, suppression of the expression of the chimeric protein has been a target in many approaches. For effective repression, RNA interference (RNAi) therapy may be a powerful tool.

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