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果蠅 dSMIF/dDcp1 與 dDcp2 去蓋頭酵素的功能分析

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行政院國家科學委員會補助專題研究計畫

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Drosophila decapping protein 1, dDcp1, as a novel component of *oskar* mRNP complex directs its posterior localization in the oocyte

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Abstract

In *Drosophila*, the posterior deposition of *oskar* (*osk*) mRNA in the oocyte is critical for pole cell and abdomen formation. Exon junction complex components, translational regulation factors and other proteins form the RNP complex essential for directing *osk* mRNA to the posterior end of the oocyte. Until now, it has not been clear whether the mRNA degradation machinery is involved in regulating *osk* mRNA deposition. Here we show that *Drosophila* decapping protein 1, dDcp1, a general factor required for mRNA degradation, is also a novel component of the *osk* mRNP complex essential for *osk* mRNA posterior deposition. During oogenesis, dDcp1 can interact with Exuperantia (Exu) in an RNA-dependent manner while *osk* mRNA is not yet set for degradation and is required for the proper localization of Exu, Ypsilon Schachtel (Yps) and Oo18 RNA binding (Orb). Clearly, dDcp1 is required for an elaborate coupling of *osk* mRNA localization in the oocyte and its degradation in the embryo.

Introduction

During *Drosophila* oogenesis, Osk is localized to the posterior end of the oocyte, nucleates polar granules, and is necessary and sufficient for abdomen and pole cell formation (Ephrussi et al., 1991; Ephrussi and Lehmann, 1992). *osk* mRNA is synthesized in nurse cells, forming a translationally repressed mRNP complex, and transported to the oocyte via ring canals (Riechmann and Ephrussi, 2001). Once inside the oocyte, an intact microtubule network, Staufen (Stau) and Kinesin heavy chain (Khc) motor protein guide *osk* mRNA to traverse the cytoplasm and reach the posterior end (Tekotte and Davis, 2002). After transportation, an actin-based cortical anchor is required to maintain the polarized distribution of *osk* (Babu et al., 2004; Baum, 2002), and, thereafter, Osk translation is derepressed.

The posterior localization of *osk* is delicately mediated by components of both the splicing and translational machinery. Exon junction complex components, such as Mago-nashi and *Drosophila* Y14, interact with eIF4AIII and Barentsz (Btz) and are required for *osk* localization (Hachet and Ephrussi, 2001; Newmark and Boswell, 1994; Palacios et al., 2004; van Eeden et al., 2001). Cup, a *Drosophila* eIF4E binding protein colocalized with both Btz and Yps, is a repressor of *osk* mRNA translation via its interaction with another translational repressor Bruno and regulates *osk* localization (Nakamura et al., 2004; Wilhelm et al., 2003). In addition to splicing and translational machinery, Exu, a *Drosophila* sponge body component, is involved in the posterior localization of *osk* mRNA (Wilhelm et al., 2000). Exu can directly interact with Yps and forms an mRNP complex containing *osk* (Wilhelm et al., 2000). Yps is a Y-box protein and antagonizes the translational stimulation function of Orb on *osk* mRNA (Mansfield et al., 2002). Orb is a *Drosophila* cytoplasmic

polyadenylation element binding protein, which mediates *osk* cytoplasmic polyadenylation and stimulates its translation (Castagnetti and Ephrussi, 2003). Orb can also interacts with Exu in an RNA-dependent manner (Mansfield et al., 2002). Although the role of Exu in *osk* mRNA localization is not yet fully understood, its interaction with Yps, and Orb implies a fine integration between the transportation and translation machineries.

Till now, it is poorly characterized whether the mRNA degradation machinery is also involved in the posterior localization of *osk*. The yeast Dhh1p, whose *Drosophila* homolog Me31B is a component of *osk* mRNP complex, can physically interacts with Dcp1p and is an activator of decapping (Coller et al., 2001). Besides, mammalian Staufen 1 (Stau1) interacts with the nonsense-mediated decay factor Upf1 and directs the decay of ADP-ribosylation factor (Arf)1 mRNA in a novel mRNA decay pathway (Kim et al., 2005). Combining that Stau is required for the posterior transportation of *osk* mRNA, it is possible that components of mRNA degradation machinery participate in *osk* mRNP complex.

Eukaryotic polyadenylated mRNA can be degraded primarily by removal of the 3' poly(A) tail, followed either by the decapping process and 5' to 3' exonucleolytic digestion, or by 3' to 5' exosome degradation. The cleavage of the 5' cap structure represents a critical step in mRNA degradation turnover (Parker and Song, 2004). The two eukaryotic conserved decapping proteins, Dcp1 and Dcp2, function together as a holoenzyme. Dcp2 has been found to be the catalytic subunit of the decapping holoenzyme but it is unclear whether Dcp1 can also have intrinsic catalytic activity. The current concept is that the primarily function of Dcp1 is to enhance the decapping activity of Dcp2 by a currently unresolved mechanism (Fillman and Lykke-Andersen, 2005; Coller and Parker, 2004).

Studies using RNAi technique indicates that dDcp1 does involve in miRNA

degradation pathway but not nonsense-mediated mRNA decay in S2 cells (Rehwinkel et al., 2005; Gatfield and Izaurralde, 2004). However, other information about the cytoplasmic localization of dDcp1 and its role during developmental processes are still unknown. Here, we first characterize *dDcp1* mutation and its protein distribution pattern. We show that dDcp1 is required for the proper degradation of *osk*, *bcd*, and *twe* in the embryo and, as a novel component of *osk* mRNP complex, it is specifically required for the posterior localization of *osk* mRNA in the oocyte. dDcp1 is colocalized with Exu in both nurse cells and oocyte cytoplasm and it is required for the proper posterior localization of Exu, Yps, and Orb. In conclusion, as a decapping factor, dDcp1 is required for not only the degradation of *osk* mRNA during embryogenesis but also its cytoplasmic transportation during oogenesis.

Results

dDcp1 is a novel posterior group gene

The *b53* mutation was recovered from a screen for homozygous lethal mutation with specific maternal effect. A P-transposase-insensitive $cFRT^{2L2R}$ chromosome (See Materials and Methods) was used as the direct target for the P{lArB} and P{PZ} mutators on X chromosome. Totally, 456 insertions on the $cFRT^{2L2R}$ second chromosome were obtained from 768 P{lArB} and 232 P{PZ} insertions. By using GLC and inverse PCR analyses, both functional and molecular descriptions of the *P*-induced lethals were delineated directly. Among 113 homozygous lethals, the P{lArB}-induced *b53* mutation was isolated from the 46 lines with specific maternal effect embryonic phenotypes.

Homozygous *b53* shows a pleiotropic lethal phase from embryo to barely survived enclosed adult. Among *b53* GLC cuticle preparation embryos, ~10% exhibit a deleted abdominal phenotype with deformed anterior structures (Fig. 1b) and the rest 90% die before cuticle formation. By inverse PCR and plasmid rescue methods, the position of the single $P\{lArB\}^{b53}$ insertion was determined (Fig. 2a). Among the 112 trasnposase-treated *b53* chromosomes, 52 revertants behave as wild-type, and this confirms that the *b53* mutant phenotype is caused by the *P* insertion. Considering the *P* insertion position, the *b53* mutation phenotype may result from the disruption of *CG11183* and/or *CG5602* (Fig. 1j). Both the *5K* and *11183L* genomic fragments covering the entire wild type *CG11183* can fully rescue the *b53* mutant phenotypes. This suggests that *CG11183* but not *CG5602* is most likely disrupted by the *P* insertion since *CG5602* is truncated in the *11183L* transgene (Fig. 1j). Unexpectedly, the *5602L* transgene containing the full length *CG5602* and the truncated *CG11183* remains able to rescue the lethality. However, the rescued *b53/b53;5602L/MKRS* flies

present a partial female sterility with posterior group embryonic phenotype (see below). We constructed another version of *5602L*, *5602L*^{BsiWI}, with a frameshift mutation after the 11th amino acid in the CG11183 N-terminal region (Fig. 1j). As expected, this *5602L*^{BsiWI} transgene can no longer rescue the *b53* lethality. This experiment excludes the possibility that *b53* mutant phenotype is caused by the *P* disruption of *CG5602* function. This is further supported by the rescue outcome of the *5602L*^{NheI} transgene, whose CG5602 coding sequence is disrupted by a frameshift mutation (Fig. 1j). Since this *5602L*^{NheI} transgene can still rescue the *b53* lethality and present a partial female sterility with posterior group embryonic phenotype (data not shown). Based on inverted excision and transgenic complementation tests using *5K*, *11183L*, *5602L*, *5602L*^{BsiWI} and *5602L*^{NheI} genomic fragments covering different lengths of the locus, we confirm that the gene disruption of *CG11183* but not *CG5602* is responsible for the posterior group phenotype of the *b53* mutation.

Database searching revealed that the N-terminal 140 amino acids of CG11183 are highly conserved with the decapping domains of decapping proteins such as Dcp1p in *S. cerevisiae* and hDcp1a and hDcp1b in human (Fig. 2a). However their carboxy-terminal regions are less well conserved (Fig. 2b). These homologues all contain a putative EVH1/WH1 domain (Fig. 2a) which is a protein-protein interaction module (Ball et al., 2002). Since CG11183 is the only Dcp1 homolog in the *Drosophila* genome, we therefore refer to it as *Drosophila* decapping protein 1 or dDcp1.

The *b53/b53;5602L/MKRS* presents a partial female sterile phenotype with a 60% unhatching rate. Among the unhatched embryos, more than 90% show a typical posterior group phenotype (Fig. 1c) and the rest 10% have a minor posterior group phenotype. In addition to the deleted abdominal phenotype, the number of pole cells are reduced (Fig. 1f, g) or totally lost (Fig. 1h, i). The *b53/b53;5602L/MKRS* genotype

is defined as the effect of the *P* insertion specifically on *CG11183*, after removing the possible effect of *CG5602* in the *b53* chromosome since the whole transcription unit of *CG5602* is included in *5602L* transgene (Fig. 1j). Besides, *5602L* transgenic flies in wild type background do not present any mutant phenotypes, which suggests that the posterior group embryonic phenotype observed in *b53/b53;5602L/MKRS* is not caused by any suspected dominant negative effects carried by the N-terminal 22kD peptide of CG11183 (Fig. 1k). This view is further strengthened by the absence of a mutant embryonic phenotype when *UASp-N180* (Fig. 1j), which produces a 180 amino acids N-terminal fragment of dDcp1, was driven by *nanos-Gal4*. Hence, the *b53/b53;5602L/MKRS* genotype providing a genetic background allows us to specifically and easily analyze the posterior group phenotype of the *b53* mutation and is renamed as the $dDcp1^{b53}$ allele.

Since there is still residual amount of full length dDcp1 protein detectable in $dDcp1^{b53}$ allele (Fig. 1k), a null allele has to be accessed. From 450 imprecisely excised P{GSV6}^{11684} chromosomes, a dDcp1 protein null allele, $dDcp1^{442P}$, was recovered as a 282 bp deletion extending from -28 to +247 bp with respect to the translation start site (Fig. 1j, l). $dDcp1^{442P}$ homozygous mutant dies at early pupal stage and its lethality can be rescued by *11183L* genomic fragment. It is difficult to examine *osk* mRNA deposition under $dDcp1^{442P}$ background since $dDcp1^{442P}$ GLC female produces early arrested egg chambers by stage 6 (data not shown). We therefore describe the *dDcp1* phenotypes based on the *b53* mutation and $dDcp1^{b53}$ allele.

dDcp1 is required for *osk* mRNA degradation

The *S. cerevisiae* Dcp1p decapping protein is a critical mediator of mRNA decay and an essential component of the 5′ to 3′ mRNA decay pathway. Loss of Dcp1p leads

to a complete block of decapping *in vivo* (Beelman et al., 1996). The function of dDcp1 in mRNA decay is supported by the genetic requirement for *dDcp1* in the degradation of *osk* and other maternal mRNAs tested. In the wild type condition, *osk* mRNA is degraded 2 hours after egg laying (AEL) (Ephrussi et al., 1991) (Fig. 3a). In contrast, *osk* mRNA is more stable and can still be detected 4 to 5 hours AEL in *b53* GLC embryos (Fig. 3a). This mRNA degradation defect is most likey caused by the defect of dDcp1 since the lethality of *b53* mutation can be rescued by the *dDcp1* wild type transgene, *11183L* (see above). Should CG5602 be responsible for the mRNA degradation defect, we would expect a complete rescue of this phenotype in $dDcp1^{b53}$ allele which contains a wild type *CG5602* transcript (Fig. 1j). However, this $dDcp1^{b53}$ allele can only partially but not fully rescue the degradation defect (Fig. 3a). This suggests that *CG5602* is not the cause. In contrast, the *11183L* transgene containing the full length *CG11183* gene (Fig. 1j) can completely rescue the delayed mRNA degradation phenotype (Fig. 3a). We have therefore confirmed that *dDcp1* mutation is responsible for the delayed *osk* mRNA degradation phenotype in *b53* GLC embryos.

The partially rescued degradation defect observed in the $dDcp1^{b53}$ allele may due to the presence of the 22kD N-terminal fragment of dDcp1 (Fig. 1k), whose amino acid sequence is highly conserved with the decapping domains in the N-terminal regions of Dcp1 protein homologues (Fig. 2a). To show that the putative decapping function of dDcp1 is required for the proper decay of *osk* mRNA, the R57 amino acid of dDcp1 corresponding to a residue previously known to be critical for Dcp1p and hDcp1a decapping activity (Tharun and Parker, 1999; Lykke-Andersen, 2002) was mutated to test its effect on *osk* mRNA degradation. This $dDcp1^{R57A}$ transgene does not present any mutant phenotype under wild type background indicating that R57A mutation does not cause any dominant negative effects. And, it can produce a comparable level of $dDcp1^{R57A}$ protein in $dDcp1^{442P}/dDcp1^{442P}$; $dDcp1^{R57A}/dDcp1^{R57A}$

ovary (data not shown) suggesting that dDcp1^{R57A} protein can be stably expressed. We therefore introduced a *dDcp1*^{R57A} transgene into the *dDcp1*^{442P} null which provides a clean background to assay if this R57A single amino acid mutation in dDcp1 still causes the *osk* mRNA degradation defect (Fig. 1j). Our results show that *dDcp1*^{R57A} transgene with *dDcp1*^{442P} null can also prolong the *osk* mRNA stability to 4-5 hours AEL as in *b53* GLC embryos (Fig. 3a). This experiment supports the idea that the decapping function of dDcp1 is responsible for the regulation of *osk* mRNA degradation in embryogenesis.

The degradation function of dDcp1 is not specific to *osk*. Both *bcd,* the anterior determinant, and *twine(twe),* the *cdc25* homologue uniformly distributed in the cytoplasm of the early embryo (Alphey et al., 1992), maternal mRNAs are degraded after 2 hours AEL in wild type (Fig. 3b, c). However, in different *dDcp1* mutant backgrounds, both *bcd* and *twe* mRNAs are not degraded properly and can be rescued by *11183L* transgene, exhibiting a pattern and time frame similar to that seen with *osk* mRNA (Fig. 3b, c). This indicates that dDcp1 seems to be a general mRNA degradation factor. Collectively, these data indicate that *dDcp1* is a novel posterior group gene required for the proper degradation of *osk* mRNA.

dDcp1 is localized at the posterior pole of the oocyte

A polyclonal dDcp1 peptide antibody recognized an expected 42kD band in a western blot of wild type ovary extracts. In *dDcp1*^{b53}, trace amounts of the 42kD band as well as an extra 22kD band were detected in ovary extracts (Fig. 1k). The 22kD band represents the N-terminal 185 amino acids of dDcp1 produced by the *5602L* transgene (Fig. 1j). The 22kD N-terminal fragment of dDcp1 and the residual 42kD full length protein may explain the partial female sterility of the *dDcp1*^{b53} allele. In *dDcp1*^{442P}, the 42kD band cannot be detected at all in a larval extract (Fig. 1l). After

stage 2 of oogenesis, dDcp1 protein is localized at the posterior pole of the oocyte until stage 7 (Fig. 4a-c). At stage 8, dDcp1 transiently accumulates at the anterior pole of the oocyte and forms a ring structure (Fig. 4d). This transient anterior accumulation pattern is similar to that of *osk* mRNA and other posteriorly localized components (van Eeden et al., 2001; Ephrussi et al., 1991; Wilhelm et al., 2000) which results from microtubule reorganization (Theurkauf et al., 1992). After stage 9, dDcp1 is relocalized at the posterior crescent and is sustained there until stage 10B, at least (Fig. 4e, f). This dDcp1 staining pattern was reconfirmed by detection of the HA-tagged dDcp1 fusion protein (Fig. 4g-i). Furthermore, the punctate cytoplasmic and perinuclear distribution pattern in nurse cells is particularly obvious, especially in early stages (Fig. 4a-c). This dynamic dDcp1 expression pattern is very similar to that of *osk*, Exu and Yps (Theurkauf and Hazelrigg, 1998; Ephrussi et al., 1991; Wilhelm et al., 2000).

dDcp1 **specifically affects the posterior deposition of** ***osk*** **mRNA**

In order to evaluate whether dDcp1 specifically affects the localization of *osk*, we examined the localization of *osk*, *bcd*, and Grk in *dDcp1* mutant backgrounds. *osk* mRNA fails to localize properly at the posterior pole in about 40% of the egg chambers produced by *b53* GLC females, and in some cases *osk* accumulates in nurse cells and the anterior pole of the oocyte (Fig. 5b). However, localization of both *bcd* mRNA (Fig. 5d; an anterior determinant (Berleth et al., 1988)) and Gurken (Grk) (Fig. 5f; a determinant of dorsal follicle cell fate (Nilson and Schupbach, 1999)) is not affected. Since both abdomen and pole cell formation are affected in *dDcp1* mutant embryos, *dDcp1* would be predicted to act upstream of *vasa* (van Eeden and St Johnston, 1999). We thus further examined the expression patterns of Stau, Osk, and Vasa in $dDcp1^{b53}$ background. In wild type oocytes, Stau, Osk and Vasa all

accumulate in a crescent that is tightly localized to the posterior pole of the oocyte after stage 9 (Fig. 5g, i, k). By contrast, in ~60% of the stage 9-10 mutant egg chambers, the posterior localization of Stau is reduced or totally diminished. In some cases, Stau stains faintly and diffusely in the middle ooplasm and shows an accumulation at the anterior cortex (Fig. 5h). Osk and Vasa staining is also dramatically reduced in the posterior ends (Fig. 5j, l). Moreover, dDcp1 mutants do not cause the precocious translation of *osk* mRNA (data not shown). Collectively these results indicate that *dDcp1* is a novel posterior group gene specifically required for the posterior deposition of *osk* mRNA.

The localization of dDcp1 is dependent on *osk* mRNA as well as on microtubule organization

Since dDcp1 is required for the proper degradation of several maternal mRNAs during embryogenesis (Fig. 3), it is possible that dDcp1 affects the posterior localization of *osk* indirectly via its decapping function. However, the R57A single amino acid mutation in the decapping domain of dDcp1 does not present posterior group phenotype in those unhatched embryos laid by the partial sterile $dDcp1^{442P}/dDcp1^{442P}$; $dDcp1^{R57A}/dDcp1^{R57A}$ females. This observation clearly rules out this possibility concerned. Furthermore, in *dDcp1* mutations, the mis-localization of *osk* mRNA could be an indirect consequence of the mis-organization of microtubule network or a direct requirement of dDcp1 for the proper *osk* mRNP transportation complex formation. To investigate if microtubule cytoskeleton orientation is affected by dDcp1, we examined the microtubule plus end using Kinesin-lacZ(Clark et al., 1994) and the minus end using Centrosomin (Li and Kaufman, 1996). In the $dDcp1^{b53}$ allele, posterior localization of Kinesin-lacZ and anterior cortex localization of Centrosomin are indistinguishable from the wild-type

(Fig. 6a-d). Overall microtubule organization and integrity were studied by examining the distribution of the microtubule binding protein, Tau-GFP (Micklem et al., 1997). In the *dDcp1*^{b53} allele the distribution pattern of Tau-GFP is indistinguishable from wild-type (Fig. 6e, f). Obviously, the requirement of dDcp1 for *osk* mRNA posterior localization is not due to an indirect effect on microtubule organization. Combing all these data, it is more likely that dDcp1 is directly involved in formation of the *osk* mRNP complex.

Stau, a dsRNA binding protein, interacts with *osk* mRNA and directs its posterior deposition (Ramos et al., 2000). It functions as an adaptor to transport *osk* in a Khc-dependent manner (Brendza et al., 2000). As suspected, dDcp1 is colocalized with Stau in the posterior end of the oocyte (Fig. 7a-c) and this colocalization is sustained until at least stage 10. In both *khc*^{27} GLC and *stau*^{D3} mutant egg chambers, dDcp1 is no longer localized to the posterior end and instead accumulates at the anterior pole and lateral cortex of oocytes (Fig. 7d, e). This redistribution behavior of dDcp1 in a *khc* mutant background is quite similar to that of *osk* mRNA (Brendza et al., 2000).

Should dDcp1 indeed be a component of the *osk* mRNP complex, its level in the posterior pole would be *osk* dosage dependent. This was proven by the excessive posterior localization of dDcp1 observed when two extra copies of *osk* genomic sequences were introduced (Fig. 7f). These data further support the hypothesis that dDcp1 is a novel component of the *osk*-Stau-Khc mRNP complex. As a component of this complex, the destination of dDcp1 should be *osk* mRNA dependent. This was shown by the anterior accumulation of dDcp1 when *osk* mRNA was ectopically localized to the anterior pole in a *osk-bcd*-3'UTR hybrid mRNA in the stage 10 oocyte (Fig. 7g) (Ephrussi and Lehmann, 1992). In both *grk*$^{2B6/HF48}$ and *par-1*$^{6821/6323}$ mutant combinations, *osk* mRNA is mislocalized to the center of the oocyte due to

misorientation of the microtubule network (Shulman et al., 2000; Gonzalez-Reyes et al., 1995). In a *grk*$^{2B6/HF48}$ mutant background, dDcp1 can be deposited in the middle of the oocyte in the same way as *osk* mRNA (Fig. 7h, i). Similarly, in *par-1*$^{6821/6323}$ mutant oocyte, dDcp1 is mislocalized and colocalized with a Kinesin-lacZ fusion protein marking the plus end of microtubules (Fig. 7j-l). Altogether, these data clearly indicate that the localization of dDcp1 is microtubule organization-dependent and determined by the position of *osk* mRNA similar to the behavior of other *osk* mRNP complex components.

dDcp1 is required for the posterior localization of Exu, Yps, and Orb

Among proteins involved in *osk* mRNA localization, Exu and dDcp1 follows a similar spatial temporal distribution pattern in both nurse cells and oocyte. In the oocyte, each transiently accumulates anteriorly and subsequently localizes to the posterior pole ((Mansfield et al., 2002). We therefore suspected that dDcp1 can interact with Exu and is a new component of the large Exu-Yps mRNP complex. This point was addressed with the GFP-Exu transgene available. As expected, dDcp1 colocalizes with GFP-Exu in the posterior pole of the stage 10 oocyte (Fig. 8a-c). Besides, the punctate pattern of dDcp1 in the nurse cell cytoplasm of stage 9 egg chambers also clearly matches that of GFP-Exu (Fig. 8d-f).

Genetically, dDcp1 is required for the posterior localization of Exu and Yps. In a *dDcp1*^{b53} background, the posterior localization of GFP-Exu after stage 9 is impaired (Fig. 8m). Instead of the wild type posterior localization, Yps accumulates excessively at the anterior pole in stage 10 *dDcp1*^{b53} oocyte (Fig. 8k, n). By contrast, their distribution patterns at earlier stages are not affected (data not shown). By co-immunoprecipitation, dDcp1 was found to interact with GFP-Exu (Fig. 8p) in an RNase-sensitive manner. This RNase-sensitive interaction between dDcp1 and Exu

indicates a complex nucleated on the RNA. Similarly, Yps can be co-immunoprecipitated with HA-dDcp1 (Fig. 8q). Combining co-immunoprecipitation and genetic results (Fig. 5 and 7), we propose that dDcp1 and Exu are in the same *osk* mRNP complex, since the interaction between Exu and *osk* was reported (Wilhelm et al., 2000). Together with the observations that dDcp1 is colocalized with Exu in both nurse cells and oocyte during oogenesis (Fig. 8a-f) and the requirement of dDcp1 for proper Exu and Yps posterior localization (Fig. 8m-o), we conclude that dDcp1 is a component of the *osk*-Exu-Yps mRNP complex.

Orb interacts with Exu in a RNA-dependent manner and functionally antagonizes Yps (Mansfield et al., 2002). We further examined whether the posterior localization of Orb is also *dDcp1* dependent. In wild type, Orb localizes to the posterior pole and anterior cortex in stage 10 oocytes (Fig. 8l). By co-immunostaining, dDcp1 was found to colocalize with Orb in the oocyte from early stages to stage 9 (Fig. 8g, h). The posterior localized dDcp1 keeps its co-localization with Orb after stage 9 (Fig. 8i), although the anterior accumulation of dDcp1 is reduced. Moreover, the posterior localization of Orb is *dDcp1*-dependent. At stage 9, dDcp1 and Orb remain co-localized in $dDcp1^{b53}$ mutant egg chambers (data not shown). In the stage 10 mutant oocyte, Orb has less posterior staining and accumulates mostly at the anterior cortex (Fig. 8o) compared with wild type (Fig. 8l). This implies that after stage 9 dDcp1 is required to recruit Orb into the posterior *osk* mRNP complex so that Orb can exert its translation activation function. Nevertheless, we cannot rule out the possibility that this Orb recruitment is *osk* mRNP complex dependent. Altogether, these results provide evidence that dDcp1 in the *osk*-Exu-Yps mRNP complex directs the posterior localization of *osk* mRNA and is genetically required for the proper localization of Exu, Yps and Orb.

Discussion

Our data reveal that the decapping factor dDcp1 is not only a novel component of *osk* mRNP complex but also required for its posterior transportation. Me31B, the homolog of decapping related factor Dhh1p in *Saccharomyces cerevisiae*, is found to be involved in the translational repression of *osk* mRNA and interact with Exu in an RNase dependent manner (Nakamura et al., 2001). Dhh1p stimulates mRNA decapping and physically interacts with several proteins including Dcp1p (Coller et al., 2001). It is likely that dDcp1 may interact with Me31B and be involved in the translational repression of *osk* mRNA. However, in the dDcp1 mutants examined, we could not observe any premature translation of *osk* mRNA (data not shown).

In current concept, it is likely that Dcp1 is able to interact indirectly or directly with every mRNA. The absence of a uniform dDcp1 staining in both oocyte (e.g. Fig. 4) and nurse cells (Fig. 8a-f) suggests that dDcp1 binds with mRNAs only when targets are set for degradation. This view is further supported by the discovery of Processing bodies or P-bodies (also referred to as GW bodies) which are specific cytoplasmic foci for active mRNA degradation in yeast and mammalian cells (Eystathioy et al., 2003; Cougot et al., 2004; Sheth and Parker, 2003). In addition to the Dcp1/Dcp2 complex, P-bodies also contain LSm complex proteins 1-7, as well as the 5' to 3' exonuclease Xrn1 (Sheth and Parker, 2003; Ingelfinger et al., 2002). Recently, the inter-conversion between P-bodies and stress granules and the movement of mRNA between polysome and P-bodies have been reported (Kedersha et al., 2005; Brengues et al., 2005). These indicate that P-bodies are dynamic structures that mRNA can leave P-bodies when its status has been changed. Although we have found that dDcp1 is co-localized with other P body components, for example *Drosophila* decapping protein 2 (dDcp2) and *Drosophila* Xrn1(Pacman), in nurse cell cytoplasm (unpublished), it is unclear whether P bodies is also resided within polar

plasm in the oocyte. In fly polar granules, Me31B, dDcp1 and the polysomal apparatus are components of the *osk* mRNP complex (Braat et al., 2004; Nakamura et al., 2001). Interestingly, their counterparts, Dhh1p/rck, Dcp1p/hDcp1a, and eIF4E do reside in yeast and human P bodies (Cougot et al., 2004; Sheth and Parker, 2003). Together with the fact that *C. elegans* Dcp2 is also present in P-granules (Lall et al., 2005), the presence of shared components suggests that P bodies and polar granules are closely related structures. This raises the possibility of an ongoing cycle in which one is converted into the other.

osk mRNA is not set for degradation until embyrogenesis (Ephrussi et al., 1991) and, presumably, dDcp1 is not yet associated with it during oogenesis. Considering the genetic requirement of *dDcp1* for the posterior localization of Exu and Yps after stage 9 (Fig. 8m-o), the dDcp1-Exu and dDcp1-Yps interactions (Fig. 8p, q) and the direct interaction between Exu and Yps (Mansfield et al., 2002), it is clear that in the oocyte dDcp1 is already associated with *osk* mRNP complex while *osk* is not yet set for degradation. Its presence is expected to cause the degradation of *osk* mRNA. But, degradation seems to be repressed and does not occur till embryogenesis. How can dDcp1 remain stably associated with its substrate without initiating mRNA decay? Firstly, the presence of dDcp1 may be independent of dDcp2. Since only a Dcpl/Dcp2 complex can be a decapping holoenzyme, Dcp1 alone can not constitute the degradation machinery (Lykke-Andersen, 2002; Lykke-Andersen, 2002; Beelman et al., 1996; Coller and Parker, 2004; Lykke-Andersen, 2002) . Secondly, Poly(A)-Binding Protein can be a repressor of mRNA decay as indicated by several observations (Coller and Parker, 2004). This point of view is further supported by the observation that the length of *osk* poly(A) tails range from 100-230 A during its transport (Castagnetti and Ephrussi, 2003). Thirdly, eIF4E and the association of ribosome on mRNA can inhibit decapping activity (Coller and Parker, 2004; Schwartz

and Parker, 2000). During oogenesis, *osk* mRNA is associated with polysomes even if it is not yet posteriorly localized (Braat et al., 2004). This suggests that a stable translational initiation complex is pre-assembled with *osk* mRNA during its transport and consequently may be able to repress decapping function of dDcp1. These mechanisms may provide a plausible explanation of an inhibitory system acting during transportation and a degradation system that can be activated or de-repressed only after *osk* mRNA is set for degradation at early embryogenesis.

Together with the findings that splicing function (Hachet and Ephrussi, 2004) and the polysomal apparatus (Braat et al., 2004) are also required to regulate *osk* mRNP complex assembly and its posterior localization, it is conceivable that components of the splicing, translation, and degradation machinery are added to the nascent *osk* mRNA step by step during its maturation. In conclusion, we propose that, with respect to developmental progression, only a complete *osk* mRNP complex including the pre-incorporation of dDcp1 can be sent to its final destination during oogenesis so as to ensure the proper degradation of *osk* mRNA in embryogenesis. The mature *osk* mRNP complex is transportation, translation, and degradation competent and the switches among these distinct functions are refined by each component of these independent but closely linked machineries.

Materials and Methods

Drosophila stocks

Oregon R (OR) was the wild-type stock used for antibody staining and generation of wild-type *Drosophila* extracts. Fly stocks were raised at 25°C on standard cornmeal and agar medium. The following transgenic stocks were used in this study: P{w+; *kinesin-LacZ* }^{KZ503} (Clark et al., 1994), P{w+; *Tau-GFP*} (Micklem et al., 1997), P{w+; *GFP-Exu*}^{NGE3} (Theurkauf and Hazelrigg, 1998), P{w+; *GFP-Mago*} (Newmark et al., 1997), *nanos-Gal4VP16* (Van Doren et al., 1998), *α4-tubulin-Gal4VP16* (a gift from N. Perrimon), P{*osk*$^{+}$6.45} (Ephrussi et al., 1991), P{*osk-bcd* 3'UTR} (Ephrussi and Lehmann, 1992), *FRT*^{42B}, P{w+; *ovo*^{D1}}/CyO (Chou and Perrimon, 1996) and *P{GSV6}*^{11684} (*Drosophila* Gene Search Project, Tokyo Metropolitan University). We also used the following mutant alleles: *stau*^{D3}, *par-1*^{6821} and *par-1*^{6323} (Shulman et al., 2000), *khc*^{27} (Brendza et al., 2000), *grk*^{2B6} and *grk*^{HF48} (Neuman-Silberberg and Schupbach, 1993). Other stocks used were provided by the Bloomington Stock Center.

The *P*-transposase-insensitive *cFRT*^{2L2R} chromosome

The *FRT*^{2L2R} chromosome, containing P{hs-neo>>, ry^{+}, FRT}^{40A} at 2L arm and P{>w^{hs}>, FRT}^{42B} at 2R arm (Chou and Perrimon, 1996), was modified to become *P*-transposase-insensitive after three consecutive *P*-transposase treatments. Based on the non-mosaic miniwhite eye color phenotype representing no somatic transposition, 427 independent *FRT*^{2L2R} chromosomes were selected after challenging twice with the *P* transposase. Among these, 107 homozygous viable lines with *rosy-minus* eye color were recovered. Quantitatively, these candidates were examined for their germ-line clone (GLC) production efficiency. The number of ovaries with developed

vitellogenic egg chambers versus total ovaries was examined according to the autosomal FLP-DFS technique (Chou and Perrimon, 1996). The two best chromosomes, with 55-85% GLC efficiency on both 2L and 2R arms, were selected for further treatment. One chromosome remained insensitive after further *P*-transposase treatment, since the GLC efficiency ranged consistently from around 55 to 85% for both arms of the ten independent homozygous viable progenies examined. The isogenized chromosome has the hatching capacity of the GLC-derived embryos higher than 95% for both arms. Apparently for this particular chromosome the repeated transposase challenge did not create detectable lesions.

Originally, P{hs-neo>>, ry^{+}, FRT}^{40A} was inserted 3' to the base T at 240696 bp of the AE003781 clone with the P3' end pointing to centromere. In *clipped* P{hs-neo>>, ry^{+}, FRT}^{40A}, imprecise excision caused the removal of P5' region and most of the *rosy*+ DNA segment, i.e. sequences from base 26 to around 2070 of P{neo FRT} , the FBtp0000348 locus (FlyBase ID), were deleted. Also, P{>w^{hs}>, FRT}^{42B} was inserted 3' to the base T at 11497 bp of the AE003789 clone with the P5' end pointing to telomere. The most P5' region and one of the FRT DNA repeats, e.g. bases 10 to 2821 of P{FRT(w^{hs})}, the FBtp0000268 locus, were deleted. Since the P5' region is necessary for transposition of the *P* transposon, both *clipped* P{hs-neo>>, ry^{+}, FRT}^{40A} and *clipped* P{>w^{hs}>, FRT}^{42B} are immobilized. Nevertheless, the FRT sequences are fully functional for FLP-driven site-specific recombination. Furthermore, after *P* mutagenesis, this chromosome permits further treatment instantly, i.e. the local imprecise excision maneuver for creating new alleles of the *P*-induced mutant generated. Due to clipping off the respective P5' regions, this new chromosome is deemed *clipped* FRT^{2L2R}, $cFRT^{2L2R}$. The genes mutated by the molecule-tagged *P*-transposon on the $cFRT^{2L2R}$ chromosome can be examined instantly for their homozygous recombinant clones by the FLP-FRT site-specific

recombination system. This chromosome is ready for systematic disruption and analysis for more than 35% of the fly genome.

Transgenes

The 5.4 kb *Nco*I-*Bam*HI *5K* fragment, the 3kb *Sal*I- *Sal*I *11183L* fragment, and the 4.3kb *Eag*I to *Bam*HI *5602L* fragment from P1 clone DS06090 (obtained from BDGP) were subcloned into pCaSpeR4 vectors for complementation tests (Fig. 1j). $5602L^{BsiWI}$ and $5602L^{NheI}$ transgenes were made by BsiWI and NheI enzyme digestion respectively followed by klenow treatment and self-ligation on the *5602L* fragment. The $dDcp1^{R57A}$ transgene with a R57A amino acid substitution on the *11183L* fragment (Fig. 1j) was generated using a QuikChange site-directed mutagenesis kit (Strategene). P{UASp-HA-dDcp1} and P{UASp-HA-N180-dDcp1} were generated by introducing the N-terminal HA-tagged full length and N-terminal 180 amino acids of dDcp1 into the pUASp vector, respectively. The dDcp1 full length coding sequence was obtained from EST clone GH04763.

Antibody generation and immunochemistry

A peptide with sequence SAPQQPKQDSSQPAS, corresponding to amino acid residues 140 to 154 of dDcp1, was used to generate polyclonal rabbit antiserum. For Western blot analysis, a 1:2000 dilution of anti-dDcp1 rabbit antibody or 5 mg/ml of affinity purified anti-Yps rabbit antibody (a gift of James E. Wilhelm) or a 1:10000 dilution of anti-α-Tubulin mouse antibody (Sigma) were used as 1st antibodies. Protein was detected by enhanced chemiluminescence using 1:15000 dilutions of HRP-conjugated goat anti–rabbit IgG (Jackson lab). For immunoflourescence staining, ovaries from 1 to 3 day-old females were dissected into PBS on ice and fixed for 20 minutes in fixative (600 ul of heptane, 200 ul of 2% paraformaldehyde in PBS,

and 1 μl of NP-40). After 3 washes with PBT (PBS plus 0.2% Tween20), the fixed ovaries were incubated in PBT containing 1% Triton-X 100 for 1 hour. Ovaries were then blocked for 3 to 5 hours in 5% normal goat serum in PBT, and incubated overnight at 4^{o}C in primary antibody diluted in PBT (1:20 for anti-dDcp1, 1:20 for anti-Orb 6H4, 1:500 for anti-Yps, 1:3000 for anti-Osk, 1:4000 for anti-Stau, 1:200 for anti-Centrosomin, 1:500 for anti-Vasa, 1:100 for anti-GFP, 1:100 for anti-HA). The ovaries were then washed 3 times for 20 minutes each in PBT, and then incubated for 2 hours at room temperature in secondary antibody in PBT. Following final 3x 30 minute washes in PBT, the ovaries were mounted in DABCO anti-fading solution (PBS containing 50% glycerol and 2% DABCO). General procedures and ovary extract preparation for co-immunoprecipitation followed those described in (Wilhelm et al., 2000), except that 500 μl of ovary extract was immunoprecipitated with either 50 μl of anti-GFP agarose (Vector) or anti-HA agarose (Sigma) for 2 hours at 4^{o}C with gentle shaking.

Northern blot analysis

Total RNAs were extracted from 1 hour interval staged embryo collections in different *dDcp1* genotypes using Purescript RNA Isolation Kit (Gentra). DIG-labeled (Roche) *osk* (744 bp), *bcd* (766 bp), *twe* (711 bp), and *rp49* (450 bp) RNA probes were made by *in vitro* transcription using DIG Northern Starter Kit (Roche). Northern blot procedures are based on “Dig Application Manual for Filter Hybridization” (Roche).

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Reference List

Alphey,L., Jimenez,J., White-Cooper,H., Dawson,I., Nurse,P., and Glover,D.M. (1992). twine, a cdc25 homolog that functions in the male and female germline of Drosophila. Cell *69*, 977-988.

Babu,K., Cai,Y., Bahri,S., Yang,X., and Chia,W. (2004). Roles of Bifocal, Homer, and F-actin in anchoring Oskar to the posterior cortex of Drosophila oocytes. Genes Dev. *18*, 138-143.

Ball,L.J., Jarchau,T., Oschkinat,H., and Walter,U. (2002). EVH1 domains: structure, function and interactions. FEBS Lett. *513*, 45-52.

Baum,B. (2002). Drosophila oogenesis: generating an axis of polarity. Curr. Biol. *12*, R835-R837.

Beelman,C.A., Stevens,A., Caponigro,G., LaGrandeur,T.E., Hatfield,L., Fortner,D.M., and Parker,R. (1996). An essential component of the decapping enzyme required for normal rates of mRNA turnover. Nature *382*, 642-646.

Berleth,T., Burri,M., Thoma,G., Bopp,D., Richstein,S., Frigerio,G., Noll,M., and Nusslein-Volhard,C. (1988). The role of localization of bicoid RNA in organizing the anterior pattern of the Drosophila embryo. EMBO J. *7*, 1749-1756.

Braat,A.K., Yan,N., Arn,E., Harrison,D., and Macdonald,P.M. (2004). Localization-dependent oskar protein accumulation; control after the initiation of translation. Dev. Cell *7*, 125-131.

Brendza,R.P., Serbus,L.R., Duffy,J.B., and Saxton,W.M. (2000). A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein. Science *289*, 2120-2122.

Brengues,M., Teixeira,D., and Parker,R. (2005). Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. Science *310*, 486-489.

Castagnetti,S. and Ephrussi,A. (2003). Orb and a long poly(A) tail are required for efficient oskar translation at the posterior pole of the Drosophila oocyte. Development *130*, 835-843.

Chou,T.B. and Perrimon,N. (1996). The autosomal FLP-DFS technique for generating germline mosaics in Drosophila melanogaster. Genetics *144*, 1673-1679.

Clark,I., Giniger,E., Ruohola-Baker,H., Jan,L.Y., and Jan,Y.N. (1994). Transient posterior localization of a kinesin fusion protein reflects anteroposterior polarity of the Drosophila oocyte. Curr. Biol. *4*, 289-300.

Coller,J. and Parker,R. (2004). Eukaryotic mRNA decapping. Annu. Rev. Biochem. *73*, 861-890.

Coller,J.M., Tucker,M., Sheth,U., Valencia-Sanchez,M.A., and Parker,R. (2001). The DEAD box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes. RNA. *7*, 1717-1727.

Cougot,N., Babajko,S., and Seraphin,B. (2004). Cytoplasmic foci are sites of mRNA decay in human cells. J. Cell Biol. *165*, 31-40.

Ephrussi,A., Dickinson,L.K., and Lehmann,R. (1991). Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. Cell *66*, 37-50.

Ephrussi,A. and Lehmann,R. (1992). Induction of germ cell formation by oskar. Nature *358*, 387-392.

Eystathioy,T., Jakymiw,A., Chan,E.K., Seraphin,B., Cougot,N., and Fritzler,M.J. (2003). The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSm4 in cytoplasmic GW bodies. RNA. *9*, 1171-1173.

Fillman,C. and Lykke-Andersen,J. (2005). RNA decapping inside and outside of processing bodies. Curr. Opin. Cell Biol. *17*, 326-331.

Gatfield,D. and Izaurralde,E. (2004). Nonsense-mediated messenger RNA decay is initiated by endonucleolytic cleavage in Drosophila. Nature *429*, 575-578.

Gonzalez-Reyes,A., Elliott,H., and St Johnston,D. (1995). Polarization of both major body axes in Drosophila by gurken-torpedo signalling. Nature *375*, 654-658.

Hachet,O. and Ephrussi,A. (2001). Drosophila Y14 shuttles to the posterior of the oocyte and is required for oskar mRNA transport. Curr. Biol. *11*, 1666-1674.

Hachet,O. and Ephrussi,A. (2004). Splicing of oskar RNA in the nucleus is coupled to its cytoplasmic localization. Nature *428*, 959-963.

Ingelfinger,D., Arndt-Jovin,D.J., Luhrmann,R., and Achsel,T. (2002). The human LSm1-7 proteins colocalize with the mRNA-degrading enzymes Dcp1/2 and Xrnl in distinct cytoplasmic foci. RNA. *8*, 1489-1501.

Kedersha,N., Stoecklin,G., Ayodele,M., Yacono,P., Lykke-Andersen,J., Fitzler,M.J., Scheuner,D., Kaufman,R.J., Golan,D.E., and Anderson,P. (2005). Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. J. Cell Biol. *169*, 871-884.

Kim,Y.K., Furic,L., Desgroseillers,L., and Maquat,L.E. (2005). Mammalian Staufen1 recruits Upf1 to specific mRNA 3'UTRs so as to elicit mRNA decay. Cell *120*, 195-208.

Lall,S., Piano,F., and Davis,R.E. (2005). C. elegans Decapping Proteins: Localization and Functional Analysis of Dcp1, Dcp2, and DcpS during Embryogenesis. Mol. Biol. Cell.

Li,K. and Kaufman,T.C. (1996). The homeotic target gene centrosomin encodes an essential centrosomal component. Cell *85*, 585-596.

Lykke-Andersen,J. (2002). Identification of a human decapping complex associated with hUpf proteins in nonsense-mediated decay. Mol. Cell Biol. *22*, 8114-8121.

Mansfield,J.H., Wilhelm,J.E., and Hazelrigg,T. (2002). Ypsilon Schachtel, a Drosophila Y-box protein, acts antagonistically to Orb in the oskar mRNA localization and translation pathway. Development *129*, 197-209.

Micklem,D.R., Dasgupta,R., Elliott,H., Gergely,F., Davidson,C., Brand,A., Gonzalez-Reyes,A., and St Johnston,D. (1997). The mago nashi gene is required for the polarisation of the oocyte and the formation of perpendicular axes in Drosophila. Curr. Biol. *7*, 468-478.

Nakamura,A., Amikura,R., Hanyu,K., and Kobayashi,S. (2001). Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during Drosophila oogenesis. Development *128*, 3233-3242.

Nakamura,A., Sato,K., and Hanyu-Nakamura,K. (2004). Drosophila cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis. Dev. Cell *6*, 69-78.

Neuman-Silberberg,F.S. and Schupbach,T. (1993). The Drosophila dorsoventral patterning gene gurken produces a dorsally localized RNA and encodes a TGF alpha-like protein. Cell *75*, 165-174.

Newmark,P.A. and Boswell,R.E. (1994). The mago nashi locus encodes an essential product required for germ plasm assembly in Drosophila. Development *120*,

1303-1313.

Newmark,P.A., Mohr,S.E., Gong,L., and Boswell,R.E. (1997). mago nashi mediates the posterior follicle cell-to-oocyte signal to organize axis formation in Drosophila. Development *124*, 3197-3207.

Nilson,L.A. and Schupbach,T. (1999). EGF receptor signaling in Drosophila oogenesis. Curr. Top. Dev. Biol. *44*, 203-243.

Palacios,I.M., Gatfield,D., St Johnston,D., and Izaurralde,E. (2004). An eIF4AIII-containing complex required for mRNA localization and nonsense-mediated mRNA decay. Nature *427*, 753-757.

Parker,R. and Song,H. (2004). The enzymes and control of eukaryotic mRNA turnover. Nat. Struct. Mol. Biol. *11*, 121-127.

Ramos,A., Grunert,S., Adams,J., Micklem,D.R., Proctor,M.R., Freund,S., Bycroft,M., St Johnston,D., and Varani,G. (2000). RNA recognition by a Staufen double-stranded RNA-binding domain. EMBO J. *19*, 997-1009.

Rehwinkel,J., Behm-Ansmant,I., Gatfield,D., and Izaurralde,E. (2005). A crucial role for GW182 and the DCP1:DCP2 decapping complex in miRNA-mediated gene silencing. RNA. *11*, 1640-1647.

Riechmann,V. and Ephrussi,A. (2001). Axis formation during Drosophila oogenesis. Curr. Opin. Genet. Dev. *11*, 374-383.

Schwartz,D.C. and Parker,R. (2000). mRNA decapping in yeast requires dissociation of the cap binding protein, eukaryotic translation initiation factor 4E. Mol. Cell Biol. *20*, 7933-7942.

She,M., Decker,C.J., Sundramurthy,K., Liu,Y., Chen,N., Parker,R., and Song,H. (2004). Crystal structure of Dcp1p and its functional implications in mRNA decapping. Nat. Struct. Mol. Biol. *11*, 249-256.

Sheth,U. and Parker,R. (2003). Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. Science *300*, 805-808.

Shulman,J.M., Benton,R., and St Johnston,D. (2000). The Drosophila homolog of C. elegans PAR-1 organizes the oocyte cytoskeleton and directs oskar mRNA localization to the posterior pole. Cell *101*, 377-388.

Tekotte,H. and Davis,I. (2002). Intracellular mRNA localization: motors move messages. Trends Genet. *18*, 636-642.

Tharun,S. and Parker,R. (1999). Analysis of mutations in the yeast mRNA decapping enzyme. Genetics *151*, 1273-1285.

Theurkauf,W.E. and Hazelrigg,T.I. (1998). In vivo analyses of cytoplasmic transport and cytoskeletal organization during Drosophila oogenesis: characterization of a multi-step anterior localization pathway. Development *125*, 3655-3666.

Theurkauf,W.E., Smiley,S., Wong,M.L., and Alberts,B.M. (1992). Reorganization of the cytoskeleton during Drosophila oogenesis: implications for axis specification and intercellular transport. Development *115*, 923-936.

Van Doren,M., Williamson,A.L., and Lehmann,R. (1998). Regulation of zygotic gene expression in Drosophila primordial germ cells. Curr. Biol. *8*, 243-246.

van Eeden,F. and St Johnston,D. (1999). The polarisation of the anterior-posterior and dorsal-ventral axes during Drosophila oogenesis. Curr. Opin. Genet. Dev. *9*, 396-404.

van Eeden,F.J., Palacios,I.M., Petronczki,M., Weston,M.J., and St Johnston,D. (2001). Barentsz is essential for the posterior localization of oskar mRNA and colocalizes with it to the posterior pole. J. Cell Biol. *154*, 511-523.

Wilhelm,J.E., Hilton,M., Amos,Q., and Henzel,W.J. (2003). Cup is an eIF4E binding protein required for both the translational repression of oskar and the recruitment of Barentsz. J. Cell Biol. *163*, 1197-1204.

Wilhelm,J.E., Mansfield,J., Hom-Booher,N., Wang,S., Turck,C.W., Hazelrigg,T., and Vale,R.D. (2000). Isolation of a ribonucleoprotein complex involved in mRNA localization in Drosophila oocytes. J. Cell Biol. *148*, 427-440.

Figure 1. Mutant phenotypes and molecular organization of the *dDcp1* gene.

a-c, Reduction of maternal *dDcp1* gives rise to an abdomen deletion phenotype. Cuticle preparations of late embryos are shown. Embryos from *b53* GLC (**b**) display a phenotype with deleted abdomen and deformed anterior region compared to wild type (**a**). Embryos from a $dDcp1^{b53}$ female (**c**) display a pure abdominal deletion phenotype. **d-i,** The number of pole cells is reduced in a *dDcp1* mutant. In $dDcp1^{b53}$ cellular blastoderm stage embryos (**f-i**) the pole cells are fewer (**f, g**) or cannot be detected (**h, i**) compared to wild type (**d, e**). Embryos were viewed by DIG (**d, f, h**), and pole cells were marked by Vasa (red) and viewed by confocal (**e, g, i**). **j,** Organization of the *dDcp1* locus at 60A8. The P{lArB}^{b53} insertion is 46 bp downstream of the transcription start site and 27 bp upstream of the translation start site of *CG11183*, and 273 bp upstream of the opposite-orientated *CG5602*. P{GSV6}^{11684} is located 35 bp upstream of P{lArB}^{b53}. The related deletion region in $dDcp1^{442P}$ is shown. The *Nco*I-*Bam*HI *5K* fragment, the *Sal*I- *Sal*I *11183L* fragment, the *Eag*I to *Bam*HI *5602L* fragment, the $dDcp1^{R57A}$ transgene, the $5602L^{NheI}$ fragment and the $5602L^{BsiWI}$ fragment used in complementation tests are shown. UASp-N180 transgene is also presented. **k-l**, Western blots of dDcp1 mutations. dDcp1 antibody specifically recognizes a 42kD band. In $dDcp1^{b53}$ ovary extract, the 42 kD band is barely visible and a 22 kD band corresponding to the polypeptide produced by the N-terminal dDcp1 from the *5602L* transgene is marked by asterisk (**k**). The 42 kD band cannot be detected in a $dDcp1^{442P}$ larval extract (**l**).

Figure 2. Sequence alignment of dDcp1, hDcp1a, hDcp1b and Dcp1p decapping proteins.

a, The N-terminal 140 amino acids of dDcp1, hDcp1a and hDcp1b are conserved with full length yeast Dcp1p. Conserved residues critical for decapping function (D18, R57 in dDcp1) in Dcp1 are marked with asterisks. Seven β sheets and an α helix that form the EVH1/WH1 domain in Dcp1p are presented (She et al., 2004). **b**, The carboxy-terminal regions of dDcp1, hDcp1a and hDcp1b are less well conserved.

Figure 3. *dDcp1* is required for proper mRNA degradation.

a-c, Northern blot analyses of *osk*, *bcd* and *twe* mRNAs in different *dDcp1* mutations. *osk* mRNA diminishes after 2 hr AEL in wild type embryos. In contrast, *osk* mRNA remains stable until at least 5 hours AEL in *b53* GLC embryos, and is partially rescued in the $dDcp1^{b53}$ allele. *osk* mRNA is maintained until at least 5 hours AEL in the R57A mutated dDcp1, $dDcp1^{442P}$/ $dDcp1^{442P}$; $dDcp1^{R57A}$/*MKRS*. This delayed mRNA degradation phenotype can be fully rescued by the *11183L* transgene in *b53/b53; 11183L/MKRS*. (**a**). Similar patterns of mRNA degradation defects and results of *11183L* transgenic rescue are observed using *bcd* or *twe* probes (**b, c**). The *rp49* mRNA is used as an internal loading control for corresponding mutations. 1 μg of total RNA extracted from embryos collected at one hour intervals was applied in each lane.

Figure 4. Distribution of dDcp1 during oogenesis.

a-f, dDcp1 antibody staining pattern. dDcp1 accumulates in the posterior of the oocyte from stage 2 and 3 (**a**), stage 5 (**b**) and stage 6 (**c**). At stage 8, dDcp1 is transiently found at the anterior end of the oocyte (**d**). After stage 9, dDcp1 is once again found at the posterior pole of the oocyte (**e**) and remains until to stage 10 (**f**). **g-i**, HA antibody staining of the egg chambers of the *nanos-Gal4VP16*-driven *UASp-HA-dDcp1* transgenic fly. The posterior accumulation in stage 2 (**g**), the transient anterior localization in stage 8 (**h**), and the posterior localization in stage 10A (**i**) are the same as the dDcp1 antibody staining.

Figure 5. Mutations in *dDcp1* specifically affect the posterior deposition of maternal determinants.

a-d, *in situ* hybridization of *osk* and *bcd* mRNA in stage 10 *b53* GLC egg chambers. *dDcp1* activity is required for the posterior localization of *osk* mRNA (**a, b**). By contrast, the localization of *bcd* mRNA is unaffected (**c, d**). **e-l**, Immunodetection of the Grk, Stau, Osk and Vasa proteins in $dDcp1^{b53}$ egg chambers. The anterior-dorsal Grk distribution in the stage 9 egg chamber (**e, f**) is not affected. The posterior localization of Stau (**g, h**), Osk (**i, j**), and Vasa (**k, l**) is reduced or lost. In some cases, a faint and diffuse Stau can be recognized within the oocyte (**h**). The cell boundary was marked by Texas-red phalloidin (red).

Figure 6. The *dDcp1*^{b53} mutation does not disrupt microtubule organization in the oocyte.

a-d, The microtubule plus end marked by kinesin-lacZ (**a, b**) and minus end marked by Centrosomin (**c, d**) are not affected in stage 9 egg chambers. **e, f**, The overall microtubule organization examined by Tau-GFP is not affected in stage 9 egg chambers (**c, f**).

Figure 7. The posterior localization of dDcp1 is *osk* mRNA dosage- and position-dependent, and microtubule organization-dependent.

a-c, HA-dDcp1 (green, anti-HA) is co-localized with Stau (red, anti-Stau) in the posterior crescent of the oocyte. **d, e,** The posterior localization of dDcp1 (green) is lost and accumulated in the anterior and lateral cortex in khc^{27} GLC (**d**) and $stau^{D3}$ (**e**) egg chambers. **f,** The posterior localization of dDcp1 (green) is increased in oocytes carring four copies of the *osk* gene. **g,** dDcp1 is ectopically localized to the anterior of the *osk-bcd 3'UTR* oocyte. **h, i,** dDcp1 (green) is mis-localized in the middle of $grk^{2B6/HF48}$ oocytes. **j-l,** dDcp1 (red) is gone from the posterior poles in $par\text{-}1^{6821/6323}$*; kin-lacZ/+* oocytes. The microtubule plus end was marked using Kinesin-lacZ (green, anti-lacZ). Stage 9 to 10 egg chambers are shown in all panels.

Figure 8. dDcp1 is required for the posterior localization of Exu, Yps and Orb and interacts with Exu and Yps.

a-f, dDcp1 (red) is colocalized with GFP-Exu (green) in the posterior end of oocytes (**a-c**) and the cytoplasm of nurse cells (**d-f**). **g-i**, dDcp1 is colocalized with Orb at the posterior end of the oocyte. Stage 5 (**g**), 9 (**h**) and 10 (**i**) egg chambers were doubly stained for dDcp1 (green) and Orb (red). **j-l**,The posterior localization of Exu (**j, m**, stage 9), Yps (**k, n**, stage 10), and Orb (**l, o,** stage 10) are disrupted in *dDcp1*^{b53} mutants (**m, n and o**). Genotype in (**m**): *b53/b53; 5602L/GFP-Exu*. **p**, GFP-Exu co-immunoprecipitates with dDcp1 in a RNase-sensitive manner. GFP-Exu ovary extract was immunoprecipitated using anti-GFP antibody. Western blot was performed using anti-dDcp1 antibody. **q,** HA-dDcp1 co-immunoprecipitates with Yps. α*4-tubulin Gal4VP16*-driven *HA-dDcp1* ovary extract was immunoprecipitated using anti-HA antibody. Western blot was performed using anti-Yps antibody. OR ovary extract was used as the control in (**p**) and (**q**). The input was 1/100 the volume of ovary extract used in the co-immunoprecipitation reaction.

參加第十屆 HUGO 國際會議心得報告

第十屆國際 HUGO(Human Genome Organization)大會於 94 年 4 月 18 日至 4 月 22 日在日本京都舉行。每年 The Human Genome Organisation 都會舉辦一個國際性的人類基因體學年度大會，藉以提升全世界相關領域的學者對人類基因體學的認識與了解。這個大會主要是提供一個激發和討論的環境，並以 plenary lectures, symposia, workshops, poster presentations 和 social events 的方式，來促使有興趣的學者專家們共同分享個人或研究團隊的研究發現和經驗；同時也讓學界和相關之生技產業得以相互溝通交流，進而達成可能之合作方案造福人群。因此，HUGO 的人類基因體學年度大會常常被認為是不可錯過的會議。今年本人有幸得以參加此次會議，特別是在人類基因體研究日趨成熟的時刻裡，和來自世界各個國家的研究團體在"From Genome to Health"的主題下共襄盛舉更是難得。此番大會之內容包括族群基因體學、醫學基因體學、功能性基因體學、癌症基因體學和最新發展出來的研究方法與技術。在醫學方面更是網羅了感染性疾病、藥物之治療、幹細胞之潛能等各方面之研究。當然也讓我們有機會見證了其他生物個體生命行為模式與人類醫學上相通且可資應用的研究訊息。關於未來醫療上必須審慎面對的醫學倫理規範也列於今年大會之課題中。

每天上午大會都邀請了國際上素負盛名的大師級基因體學專家， 就現階段各相關領域之努力與成果給與會人士一個整體性之報告。 在四天緊湊的安排中，我最感興趣的課題是如何將基因體學得到的這些訊息應用於人類之疾病研究上。過去十年來全球各地的科學家們戮力完成人類基因的序列和解讀生命之書，人類遺傳學家更是忙碌於研發新的分子生物技術來增進臨床醫師們的診斷基礎。如今我們已有超過 1500 個疾病基因之記載，在大多數的報告中均指出致病基因的突變，常常是關係著基因產物-蛋白質的改變或異常。然而這些分子在生命個體中之作用，也不是單純的幾種機制在控制著正常基因的功能和病變。因此人類基因體學提供了一個新的理念，那就是基因體的整體表現，讓我們更能切入每個基因在整個生命個體中所扮演之角色。我們可以同時偵測許多基因的異常來估計造成一個疾病的風險程度，然而我們也同樣面臨了如何去整合這些資料和其他不屬於基因層次的因素，來判定一個疾病所產生的臨床徵狀和他們的關聯性。這其中包含了生命個體本身之差異、體內細胞構造或是功能上的恆定性和適應性，而我們所認知的蛋白質便是傳遞基因訊息的最好媒介而且用於展現生物個體特有之專一性。幸運的是，我們人類已經有能力來鑑識個體間之基因差異，並且可以將之應用於個人化醫學和預防醫學上。

目前，在人類基因體學的研究中最被寄以厚望的便是基因序列上所呈現的單一核苷酸多型性，它的數目眾多(大約有五百萬個)，而且它們分散在人類之基因體各處，很適合作為個人化醫學和人類複雜疾病表現之基因定位之用；同時，

由於它的出現頻度會因為不同族群而呈現差異性，因此族群遺傳學的研究也提供了未來各族群間醫療資訊互通前一個非常重要的指標。針對單一核苷酸多型性的應用，一個集鉅資與大量高承載、高產能的世界級研究組織團體已於 2003 年成立，並如火如荼的展開各項測試，我們已經在本年度的人類基因體學大會中看到一些成績了。另外，人類遺傳學家也注意到其他可供我們解釋很多生命現象的其他基因體上的資源，它們可以用基因之表現度、基因之剪接和基因之標記來調控一些特殊之生物功能。這些結果也都可以在一些人類疾病中得到驗證。再者，為因應未來之醫學發展和個人化醫學之施行，英國和日本已經開始籌建屬於他們族群特有之生物銀行，目標希望能夠繼續找出一些常見疾病的致病基因，尋求新的藥物治療和診斷方法。

此次大會，因為日本為主辦國而且一直在人類基因體學研究各方面素有成績，表現得很讓人另眼相看。台灣方面共來了十來位學者專家，大都有發表論文，個人在此方面仍屬學習階段，但是收穫很多。