# THE ROLE OF GENE *DnudC* IN A TUMOR SUPPRESSOR GENE IN OOGENESIS

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The Drosophila *nudC* gene (*DnudC*) is a homolog of the fungal nuclear distribution gene C (*nudC* gene) in *Aspergillus nidulans.* This gene was first identified in this fungus during a screen for temperature sensitive mutations that severely affected nuclear distribution but did not perturb nuclear division. In this organism, *nudC* was found to be required for regulation of nuclear movement, colony growth, cell wall deposition and viability (Cunniff *et al*., 1997; Morris, 2000; Osmani *et al*., 1990).

Further genetic studies of nuclear migration in fungi have resulted in the identification of several key nuclear distribution (*nud*) genes and their corresponding proteins (Morris, 2000). The characterization of *nud* mutants in *A. nidulans* that prevent nuclear migration into the mycelium revealed that the cytoplasmic dynein-dynactin complex is a major contributor to these processes (Morris, 2000). Their mammalian counterparts appear to be components of the cytoplasmic dynein-dynactin complex or regulators of the dynein function (Morris, 2000; Wynshaw-Boris and Gambello, 2001). The *nudC* gene has also been associated with the microtubule motor dynein-dynactin complex (Morris, 2000; Aumais *et al*., 2001). Xiang *et al*. (1995)

proved that the *nudC* mutation in *A. nidulans* induces the reduction of the protein level of NudF at restrictive temperature. Additionally, Aumais *et al*. (2001) have shown that mammalian NudC associates with lissencephaly 1 Lis1 (NudF) and the dynein-dynactin complex. These two results suggest that *nudC* and *nudF* are likely to regulate the same function. Recently, Helmstaedt *et al*. (2008) showed that the conserved *NudF* and *NudC* proteins play a concerted role at spindle pole bodies at different stages of the cell cycle in *A. nidulans*. However, the same interaction has not yet been proved in *Drosophila* and the precise mechanism of action of the *nudC* gene in this organism and its corresponding function in mammals remains largely unknown.

The cytoplasmic dynein-dynactin complex is involved in many fundamental cellular processes, including among others, nuclear migration, mitosis, motility and intracellular trafficking (Karki and Holzbaur, 1999; Vale, 2003). During mitosis, this complex has been implicated in several essential events like the formation and organization of mitotic spindles, the alignment and segregation of chromosomes and the regulation of microtubule dynamics (Karki and

Holzbaur, 1999; Mountain and Compton, 2000).

In *Drosophila*, there are several well known examples of nuclear migration during development. The first occurs during oogenesis when the oocyte nucleus undergoes a displacement from the basal to the apical end of the developing egg chamber. This nuclear movement is critical for establishing the egg and the embryonic axes, consistent with the observation that nuclear migration is a microtubule-dependent process. This apical migration is thought to be triggered by the re-orientation of the microtubule network. A second example takes place during embryogenesis, when the dividing nuclei of the syncytium migrate to the cortex also in a microtubule-dependent process. A further example is the differentiation of the individual cell types, which makes up the ommatidia of the adult eye. This process consisting of the movement of a morphogenetic furrow induces nuclear migration in the eye imaginal disc during the third larval instar. However, it is not known whether it is a microtubule dependent process yet (Cunniff *et al*., 1997).

*DnudC* encodes a 331 amino acid protein, with a molecular weight of 38.5kDa. The coding region is composed of five exons interrupted by four introns. *DnudC* is a single copy gene that maps to the left arm of the third chromosome at polytene band position 73C1 (Cunniff *et al.,* 1997). *NudC* has also been proven to be a highly conserved protein throughout

evolution, particularly in the carboxy terminal region where the function may be encoded (Aumais *et al*., 2003). The high degree of *nudC* gene and protein conservation - both at the structural and the functional level - between diverse organisms suggests that this gene has been retained for a critical function, likely involving nuclear movement and cell division (Gocke *et al*., 2000).

In mammals, *NudC* is associated with proliferative capacities. Immuno-histochemical examination of human tissues has provided evidence that *HnudC* has a role in eukaryotic cell proliferation (Gocke *et al*., 2000). Furthermore, *HNudC* also appears to be involved in a proliferation-related manner in malignant cell growth (Miller *et al*., 1999). Data from Miller *et al.* (1999) demonstrated that *HNudC* is significantly enhanced in leukemic cells and tumours. This evidence suggests that the upregulation of the expression of *HNudC* may be part of the onset of human cancer.

It has been proved that *NudC* is a mitotic phosphoprotein, whose phosphorylation correlates with stages of the cell cycle that have elevated Polo-like kinase 1 (Plk1) activity (Nishino *et al*., 2006; Zhou *et al*., 2003). The close interaction between these two proteins suggests that *NudC* is responsible (at least in part) for the unique subcelluar localization of Plk1 (Nishino *et al.,* 2006).

Polo-like kinase 1 (Plk1), plays essential roles at multiple events during cell division, as many other kinases (Nigg,

1998). Plk1 localizes to centrosomes at interphase and prophase, the spindle poles at metaphase, the central spindle during anaphase, and the midbody during cyto-kinesis (Golsteyn *et al*., 1995). These localizations contribute to Plk1's specific functions during mitosis and cytokinesis. Thus, the correct localization of polo-like kinase is essential for its mitotic functions (Lee *et al*., 1998; Song *et al*., 2000).

This study aims at investigation the validity of Piggy-Bac insertion mutation in *DnudC* gene, the mutant phenotype(s) using somatic and germ-line clone assays, and determination of *DnudC* expression patterns during embryo development, third larvae instar, and oogenesis.

## MATERIALS AND METHODS

### Materials

### Drosophila stocks

l(3)PL00734 mutant line with a genetic structure of *y w; nudCBac{3xP3-EYFP, p-Gal4D-K10}P{FRT(w[hs])}2A, P{neoFRT}82B/TM3 Ser,* and Hermes transposon (jump-starter, J10) line, *w[l*]; Her{3xP3-ECFP, atub-piggyBac-K10}M10.III*, were offered by Hacker mainwhile, the following stocks were kindly provided by the Bloomington.. Two strains, *P {ry+; hsFLP}, y w[1118]; Dr/TM, Sb* (source of FLP recombinase) and *w; P {w[+mC]=Ubi GFP.D} 61EFP{ry[+t7.2] = neoFRT}2A /TM₃* were used for somatic clones. A deficiency line w[1118]; Df(3L)Exel9002, P+PBac{XP5.WH5}Exel9002/TM6B, Tb[1] was used for complementation test.

Males of *a* dominant female sterility line( Bloomengton line # 2139) *w[*]; P{w[+mC]=ovoD1-18}3L P{w[+mW.hs]=FRT(w[hs])}2A/st[1] betaTub85D[D] ss[1] e[s]/TM3, Sb[1]* was used for Germ-line clones. *w; Dr/TM₃, Sb* strain was used to balance and select the mutated chromosomes. Genetic symbols, genetic nomenclature, gene names, and cytology are according to Lindsley and Zimm (1992), FlyBase (flybase.bio.indiana.edu; Fly-Base Consortium, 1999). All of these stocks were obtained from the Bloomington Drosophila Stock Center (http://flystocks.bio.indiana.edu).

### Culture conditions

All fly stocks and crosses were maintained and grown on standard medium of corn meal, agar, yeast, and sucrose, supplemented with dried live yeast. Flies were maintained at 18°C, while, crosses and other phenotypic assays were done at 24±1°C. All crosses were repeated at least twice.

### Methods

### Somatic mosaic analysis

The $F_1$ mosaic screens were performed as described by Xu and Harrison (1994) with some modifications. Somatic clones were generated by using *hs-FLP* recombinase carried on the first chromosome *(y, w, P[hs-FLP])*. Clones were induced in flies of the genotype *(y w, P[hs-FLP]; nudCBac{3xP3-EYFP, p-Gal4D-K10}P{FRT(w[hs])}2A, P{neoFRT}82B / P {w[+mC]=Ubi*

*GFP.D} 61EFP{ry[+t7.2] = neoFRT}2A,* by exposing larvae to a 37°C heat-shock for 1 h for two successive days, followed by recovery and incubation at 25°C until eclosion. As controls, clones were produced in parallel from chromosomes that carried the FRT alone and from non-heat-shock animals. An average of 500-700 flies of resulting $F_1$ treated larvae were screened for the presence of somatic clones with a tumor phenotype under stereomicroscope (25X to 100X). Different phenotypes were scored and photographed documented.

### Lethal phase determination

The line l(3)PL00734 was balanced over TM6B;Tb to make identi-fication of homozygous flies more easier. Eggs were collected from heterozygous mutant flies once a day for five days on 'egg-lay' vials supplemented with yeast paste. The development of the progeny was monitored, and the presence of homozygotes was recorded during all viable developmental stages. Homozy-gous mutants were identified by their wild-type body length, while heterozy-gotes larvae were identified using *Tb*. The lethal phase was assigned to a develop-mental stage in which homozygous mutant larvae appeared last (taking into account that the length of this devel-opmental stage could be extended with respect to a wild type stage).

### Complementation test

For the complementation test of *DnudC* mutant the deficiency w[1118];

Df(3L)Exel9002, P+PBac{XP5.WH5}Exel9002/TM6B, Tb[1] with breakpoints of 73D1;73D1 (Moberg *et al., 2004*) was used to verify that the inserion is responsible for the mutation.

### In situ hybridization

*In situ* hybridization to whole-mount embryos, ovaries, imaginal discs and brain using DIG-labelling (Boe-hringer Mannheim) and both sense and antisense *nudC* RNA as a probes, was performed essentially as described by Tautz and Pfeifle (1989). In case of *in situ* hybridization on ovaries, imaginal discs and brains, the hybridization protocol without proteinase K using 2 µl of *nudC* sense and antisense RNA probes was applied. The imaginal discs and the brains were dissected in PBT from well fed third instar larvae; fine dissection was carried out only when mounting. The ovaries were dissected in PBS on ice from 5 to 7-day-old well fed females; ovariole dissection was carried out when mounting.

### RNA probe

Labeled RNA probes were prepared by transcribing DNA *in vitro*. Sense and antisense probes can be generated from the same vector (knowing the direction of the gene). The sense strand can be used as a control for nonspecific staining. For this report, the vector was used to amplify the corresponding genes of interest by antisense transcription using T3

polymerase (1μg DNA template, 2μl labelling mix, 2μl transcription buffer, 1μl T3 polymerase, 0.2 μl RNase out and dH$_2$O up to 20 μl) at 37°C for 2h.

### RT-PCR

Molecular analysis. Construction of *pBac{3xP3-EYFP, p-GAL4△-K10}*, and *pHer{3xP3-ECFP, αtub-piggyBacK10}* is described in detail in Horn *et al.* (2003). DNA sequences flanking recessive lethal *piggyBac* transposon insertion was amplified by inverse PCR as described (Huang, *et al.*, 2000). In brief, five fly equivalents of genomic DNA were cleaved with *Hae*III and ligated. Flanking sequences were amplified by PCR [5 min, 95°C; 35x (30 s, 95°C; 1 min, 65°C; 2 min, 72°C); 7 min, 72°C] by using primers PLF (5'-CTTGACCTTGCCACAGAGGACTATT AGAGG-3') and PLR (5'-CAGTGACACTTACCGCATTGACAA GCACGC-3') for the 5' junction and PRF (5'-CCTCGATATACAGACCGATAAAAC ACATGC-3') and PRR (5'-AGTCAGTCAGAAACAACTTTGGCA CATATC-3') for the 3' junction. Amplified DNA fragments were directly sequenced by using primers PLR and PRF, respectively. Sequences were analyzed by using BLAST searches of the *Drosophila* Genome Database at www.ncbi.nlm.nih.gov/BLAST.

### Production of germline clones

*nudC* homozygous germlines clones were generated with the FLP/DFS

techniques as described by Chou and Perrimon (1996). Larvae were heat-shocked twice at 37°C for 60 min during third instar larval period. Three to five days old virgin females of the appropriate genotype *(y, w, P[hs-FLP]; nudCBac{3xP3-EYFP, p-Gal4D-K10}P{FRT(w[hs])}2A, P{neoFRT}82B / P{w[+mC]=ovoD1-18}3L P{w[+mW.hs]=FRT(w[hs])}2A)* were crossed to l(3)PL00734 mutant males and allowed to lay eggs for 3 days. Control crosses were tested in parallel by using appropriately marked females *(y, w, P[hs-FLP]; P[ry$^+$, hs-neo, FRT]2A, P[y$^+$], Sb/ P{w[+mC]=ovoD1-18}3L P{w[+mW.hs]=FRT(w[hs])}2A).*

### Examination of mosaic ovaries

For Immunohistochemistry, Fixed ovaries were stained as described by Cummings and Cronmiller (1994) with two different primary antibodies (1:2000 Vasa and 1:60 Polo) and with rhodamine-conjugated phalloidin (1:60) (Molecular Probes). The fluorescence-conjugated secondary antibodies were added together with DAPI. Goat anti-rabbit Cy3 (1:1000) was used to detect Vasa and goat anti-mouse Cy3 (1:1000) was used to detect Polo; meanwhile, DAPI was used at 1:1000.

Ovaries of *(y, w, P[hs-FLP]; nudCBac{3xP3-EYFP, p-Gal4D-K10}P{FRT(w[hs])}2A, P{neoFRT}82B / P{w[+mC]=ovoD1-18}3L P{w[+mW.hs]=FRT(w[hs])}2A)* females which did not produce eggs and wildtype ovaries were prepared for examination. At

5-7 days post-eclosion, ovaries were dissected, fixed in 4% paraformaldehyde and were washed with PBT (PBS and 0.1% Triton X-100) 5 times for 15 min each wash. The ovaries were incubated in 0.5% goat serum diluted with PBT for 1 hour. Appropriate primary antibodies were added to PBS and incubated at 4°C overnight, then washed with PBT for 5 times for 15 min or more each wash. Lastly, appropriate secondary antibodies were added and incubated overnight, then washed with PBT 5 times for 15 min each wash. After the last wash, the stained ovaries were mounted in Vectashield mounting media (Vector). Stained ovaries were visualized in all cases on Zeiss Axiophot/Axioscope microscopes, for fluorescent an appropriate excitation filter was selected and images were captured using digital cameras.

## RESULTS AND DISCUSSION

### Somatic clone phenotypes of DnudC mutant

*DnudC* mutant line (l (3) PL00734) is a *piggyBac* insertion mutation. It has been isolated during the screen for new tumour suppressor genes using FLP/FRT mosaic system in Drosophila. In somatic clone assay, homozygous mutation of *DnudC* showed many different abnormal overgrowths, necrotic and melanotic tumours on heterozygous background of adult flies (Fig. 1).

Induced somatic clones using FLP/FRT system showed survival rate reduction of emerged heterozygous adult flies, which carry the *DnudC* mutant and FRT chromosomes due to severe melanotic tumors in late third inatar larvae and pupal stages. Many larvae have large internal melanotic tumors in different locations depending on the site of the induced homozygous clone and they didn't success to complete the metamorphosis and die either as larvae or in pupal stage, as shown in Fig. (1A and B), a large melanotic tumor and several melanotic nods in the small lobe of lymph gland. Most of wing tumors were necrotic ones, which affect the whole wing blade and small part of notum (Fig. 1C), and the magnification in Fig. (1E) and it sometimes appears as an undifferentiated mass of tissue (Fig. 1F and magnification as in 1G). Somatic clones with tumor activity were observed on head (Fig. 1C and D) and on the male genitalia (Fig. 1J and K) induced massive cooperative overgrowth of cells to form unpatterned, overproliferated tissues with many centers or lobes. In addition, small patches of melanotic tissue were noticed on legs (Fig. 1H) as well as large melanotic into the abdomen (Fig. 1I). However, in some somatic clones, loss of tissues was a remarkable phenotype in eyes and wings (data not shown) which might reflect apoptosis induction.

The somatic clones phenotypes indicated that *DnudC* gene is very important in some biological process during larval stages and metamorphosis such as hematopoiesis and it has tumor suppressor activity through controlling of cell proliferation and differentiation.

Black melanotic spots are found in a number of different mutants and have been called, interchangeably, melanotic tumors or pseudotumors. These "tumors" are usually not invasive and involve tumorous overgrowth only in some instances (Watson *et al.*, 1994). Minakhina and Steward (2006) reported that the melanotic masses can be subdivided into melanotic nodules engaging the hemocyte-mediated encapsulation and into melanizations that are not encapsulated by hemocytes. Encapsulated nodules are found in the hemocoel or in association with the lymph gland, while melanizations are located in the gut, salivary gland, and tracheae. *DnudC* homozugous mutation clones showed both melanotic tumor phenotypes. This result is in agreement with those of Minakhina and Steward (2006) who found many mutations of genes such as *Pr- Set7*, *skpA*, and *Su(var)205,* involved in chromatin and chromosome structure, which show the highest divergence of melanotic tumor phenotypes.

In general, *DnudC* mutation revealed the highest divergence in somatic clone phenotypes in larvae and adults not only in melanotic tumor but also in overgrowth tumors and apoptosis. This result indicates the tissue-specific role of *DnudC* gene. However, FLP/FRT system, used to identify many of Drosophila genes that regulate cell proliferation, cell size and apoptosis (Xu *et al*., 1995; Ito and Rubin, 1999; Gao *et al*., 2000; Tapon *et*

*al*., 2001 and 2002) is a good sensitive system for these purposes.

### DnudC, a piggyBac insertion in the first intron

This mutant line is one of a collection of *piggyBac* insertion mutations, which was not included in the original article published by Häcker *et al.* (2003). To analyze and confirm the molecular lesion and what gene has been interrupted in this mutant, the DNA flanking sequence of *piggyBac* insertion was determined using plasmid rescue. The obtained sequence was investigated using BLAST database and the result revealed that the *piggyBac* insertion in this mutant line (l (3) PL00734) is disrupting the first intron of *DnudC* gene (Fig. 2). The gene of *DnudC* has been proven to consist of 5 exons and 4 intros in a total genomic region of about 20 kb (Cunniff *et al*., 1997). The localization of the insertion suggests that this null *nudC* mutation might be the consequence of an aberrant splicing that likely leads to a very short NudC protein.

Furthermore, it is important to mention that the mutator *piggyBac* element is marked with 3xP3-EYFP which is a highly sensitive transformation marker in the eyes and the central nervous system (Häcker *et al*., 2003). This dominant fluorescent marker serves as a visible label for the insertion in both larval and adult stages and therefore facilitates stock keeping.

## Tumour phenotype and reversion to wildtype

*DnudC* homozygous embryos hatched normally and the first instar larvae looked indistinguishable from their heterozygous siblings. At the second instar larvae (L2) stage, the development of the homozygote larvae was clearly retarded and mutant larvae died 4-5 days later with a clear necrotic over-proliferation phenotype in the salavery glands, head and cuticle (Fig. 3a-c). The survival of the *DnudC* mutants through embryonic and early larval development is probably due to the contribution of maternal *DnudC* protein, as will later be demonstrated in the germline cloning assay. This line was classified as a larval stage lethal line.

The lethal phenotype of the homozygous *DnudC* indicates that this gene is likely to code for essential functions, required for the viability of the organism.

Furthermore, to directly correlate the insertion with the mutant phenotype, the chromosome bearing the lethal mutation (3L) was re-exposed to a transposase by crossing it to the strain which carries a *hermes* transposase according to Horn *et al.* (2000). Reversion of the lethal phenotype to wild type by the excision of the *piggyBac* element was detected by the appearance of viable homozygous wild type adults, which lack both of yellow fluorescent protein (EYFP) and enhanced cyan fluorescent protein (ECFP), as dominant markers of *piggyBac* and hermes, respectively, in a subsequent generation. For the intron-insertion line PL00734, the excision of the *piggyBac* transposon has been proven to rescue the *nudC* recessive lethal phenotype. This proved that the lethality was indeed a consequence of the *piggyBac* insertion, which is of great importance for the further characterization of the function of the gene.

## Complementation test

Complementation test was used for further characterization of the mutant chromosome. The mutant line PL00734 was crossed to a deficiency stock where group of genes were deleted around the 73D area (Table 1). The deficiency failed to complement the mutation in *DnudC* and the heterozygous larvae for deletion and *DnudC* mutation showed the same phenotypes of homozygous mutant. This result indicated that there is only one lethal insertion associated with this chromosome.

This result together with the reversion to wild type as mentioned before in the previous section, confirm that the mutation in *DnudC* is the only reason for the recessive lethal phenotype associated with this line.

## Expression pattern of the DnudC gene

To study the developmental stages at which *DnudC* is expressed, whole-mount *in situ* hybridization of ovaries, embryos and third instar larvae imaginal discs and brain were performed (Fig. 4). The spatial distribution of the *DnudC*

transcript was determined using digoxigenin-labeled RNA probes derived from the coding region of the gene. Whole mount *in situ* hybridization to embryos revealed that *DnudC* is ubiquitously expressed during cleavage (A) and early embryonic developmental stages (B) suggesting the presence of a maternal contribution. At stage 9-10 (C), the staining is localized in the presumptive midgut and the proctodeal invagination. Around stage 13 (D): the expression is enriched around the gut (possibly the area corresponding to the anterior and posterior midgut), the peripheral nervous system (PNS) and some anterior sensory organs. At stage 16 (E), there is a clear and strong straining in the gut area which is likely to be the visceral mesoderm (around the gut or in the gut itself), the staining itself is more prominent in the posterior than the anterior part. A different plane of focus of the same specimen (F) shows enrichment at the PNS. Around the same stage, but from a dorsal view (G), there is again a clear straining in the PNS, the visceral mesoderm and the anterior sensory organs. Different plane of focus on the last picture (H) shows *DnudC* RNA expression in the PNS but not in the central nervous system (CNS).

The expression pattern was also examined during oogenesis where high levels of *DnudC* RNA were observed in the ovary. It was observed that there was an expression in egg chambers at all stages (I), both in the somatically derived follicle cells and in the germ line. *DnudC*

message is enriched and accumulates at higher levels in the cytoplasm of nurse cells. On the contrary, the oocyte shows low RNA expression which can be attributed to the fact that the probe does not easily penetrate the oocyte. A magnification of the anterior part of the ovariole (K) highlights that the most anterior tip of the germarium is not stained. An anti-sense probe (J) was included as a control for the specificity of the probe.

Finally, *DnudC* gene expression was also studied in the brain and imaginal discs from third-instar larvae. In this case, a slight enrichment in the periphery of the leg imaginal disc (L) and the wing imaginal disc (N) could be observed. It was expected to obtain high levels of expression in the eye imaginal disc as a consequence of documented nuclear migration activity in this organ (Cunniff *et al*., 1997); however, the obtained results of *in situ* analysis did show this (data not shown). The same applies for the brain. This organ was also expected to show high levels of expression due to reported high expression of *HnudC* in human brain tissues and a suggested role in neuronal migration and human brain development (Aumais *et al*., 2001; Matsumoto and Ledbetter, 1999). However, our analysis shows that in the third instar larval brain (M) there is no *DnudC* mRNA expression. This suggests that the *Drosophila* homologue *DnudC* might have a different function. On the other hand, one could argue that these results are the consequence of a limited capacity of this technique to efficiently stain the

brain tissue. In order to refute this option, one should try to determine the expression distribution by immunolocalization using antisera against *DnudC* in the larval brain.

The fact that *DnudC* is ubiqui-tously distributed in the developing egg chambers and the early embryo suggests that it might be involved in the reported processes of egg chamber and syncytial embryo nuclear migration (Cunniff *et al*., 1997).

### DnudC maternal effect in inhibition of germline proliferation during early oogenesis

The early expression of *DnudC* mRNA during embryogenesis suggests that the message is maternally contrib-uted. Furthermore, in a Northern blot analysis performed by Cunniff, *et al*. (1997), the *DnudC* transcript was reported to be present throughout *Drosophila* development, and to be much more abundant in adult female flies than in adult males. Their data showed that the message might be transcribed in the ovary. This result is in agreement with the high levels of expression that we have found in the cytoplasm of nurse cells, at late stages of egg chamber development. The expression pattern of *DnudC* during oogenesis and early embryogenesis, suggests that this gene may have an important function in the ovary and embryo. To investigate this possibility, mosaic egg chambers, whose germline lacked wildtype *DnudC*, were produced using the FLP-DFS technique (Chou and Perrimon, 1996).

The germline clones failed to produce any eggs. Staining of the dissected ovaries with DAPI and rhodamine-coupled phalloidin (Fig. 5A) revealed a string-like phenotype, related to a possible growth arrest of germline stem cells or cystoblasts. Such mitotic inhibition appears to happen even before the early arrest of oogenesis linked to the $ovo^D$ dominant marker. This phenotype is consistent with the results of previous studies, which demonstrate that nudC downregulation inhibits cell proliferation (Aumais *et al*., 2003; Gocke *et al*., 2000; Miller *et al*., 1999). These findings suggest that *nudC* is required for germline development at the very first moments of oogenesis.

A similar phenotype was previously reported by Bellotto *et al*. (2002), during a germline cloning screen for P-element lethal insertions. Their data revealed that the phenotype was the consequence of the absence of germ cells. In their *agametic* mosaic ovaries, the somatically derived follicle cells col-lapsed and appeared as a string-like structure. However, either early oogenesis cell division arrest or the absence of germ cells, gives a similar phenotype because they basically refer to the same problem: namely inhibition of germline develop-ment during normal somatic cell development.

Furthermore, the occurrence of a few egg chambers, which was detected (Fig. 5B) with large numbers of what is likely to be small undifferentiated germ

cells (a phenotype similar to the one related to ovarian tumours). However, further molecular characterization, using specific antibodies, will be required to verify such hypothesis, and to reveal the possible processes involved in the onset of the above mentioned phenotypes.

Double staining of egg chambers with anti-wasa and anti-polo antisera failed to give any specific staining for the study of the expression of Wasa and Polo within the *nudC* clones (data not shown), these may be due to the absence of nudC protein which is essential for localization of Polo to the centromeres as described by Nishino *et al.* (2006). In general, the obtained results demonstrate that *DnudC* is maternally contributed, and that it is likely to function as an essential regulator of cell proliferation during oogenesis.

## SUMMARY

Intracellular nuclear migration participates in the establishment of cell polarity, in asymmetrical cell division, in karyogamy and a myriad of other cellular important functions. It appears to play a role in tumor cell migration, metastasis and human brain development. In this study, the *Dnud*C gene a *Drosophila* homolog to *Aspergillus nud*C gene and its implication in tumor suppression function was characterized. The results of this study indicated that *DnudC* is expressed during development of embryos as well as during larval stages and adults. The mutation of this gene indicated that the product(s) of DnudC gene is an essential for survival during early larval stages, where, homozygous larvae die in 2<sup>nd</sup> instar larvae with many melanotic tumors as well as is essential for oogenesis. Further more, loss of heterozygousity for this mutation induce overproliferation in larval and adults as indicated by somatic clone assay using FLP/FRT system. The product of *DnudC* gene is maternally supplied in egg and this product(s) is needed in early developmental stages during development of embryos.

## ACKNOWLEDGMENTS

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Table (1): Complementation test *DnudC* was mapped with respect to deficiency Df(3L)Exel9002. The deficiency fails to complement the mutant line PL00734.

| Line | Gene | Cytology | Df(3L) tested | Complementation |
|------|------|----------|----------------|-----------------|
| PL00734 | *CG9710, DnudC* | 73D1 | w$^{1118}$; Df(3L)Exel9002, P+PBac{XP5.WH5}Exel9002/TM6B, Tb$^1$ | No |
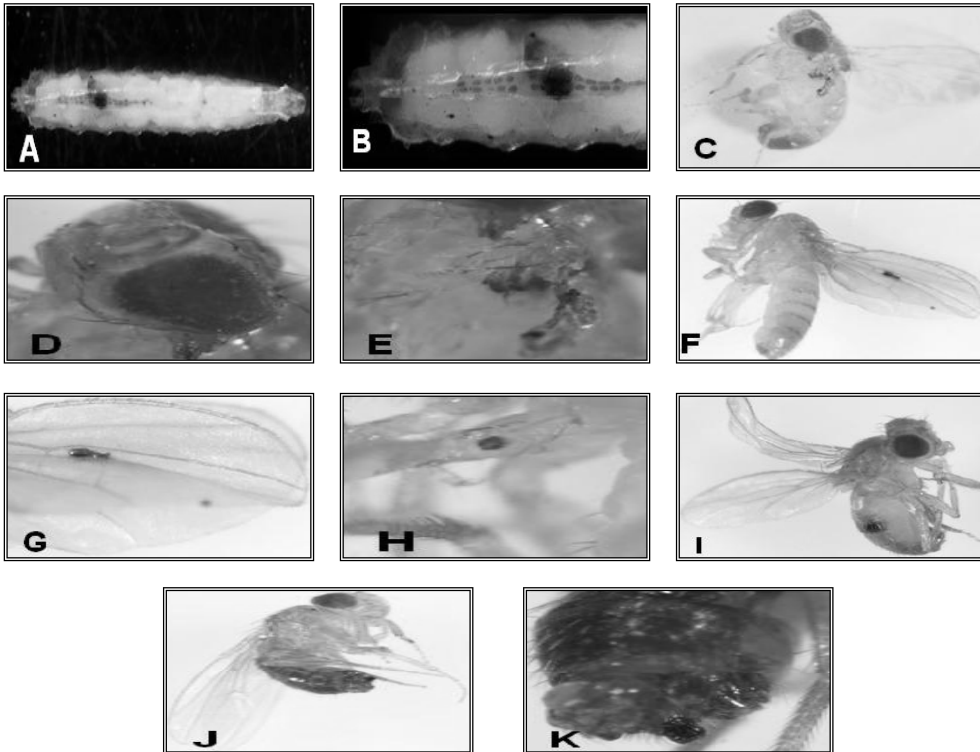


Fig. (1): Induced somatic clone phenotypes in heterozygous *DnudC* mutant larvae and adult flies of *Drosophila melanogaster* using FLP/FRT system after two hours of heat shock on two successive days in late second and early third larval stages:
(A) Large melanotic tumor with several melanotic nods in the small lobe of lymph gland. (B) magnification of A, (C) a male fly carries necrotic wing tumor affect the whole wing blade and a head tumor pops up down the eye, (D) magnification of a head tumor in C, (E) magnification of a wing tumor in C, (F) an undifferentiated mass of tissue on the wing, (G) magnification of a wing tumor in F, (I) a large melanotic tumor into the abdomen, (J) unpatterned overgrowth of cells on the male genitalia, (K) magnification of overproliferated tissues with many centers or lobes of male genitalia in J.
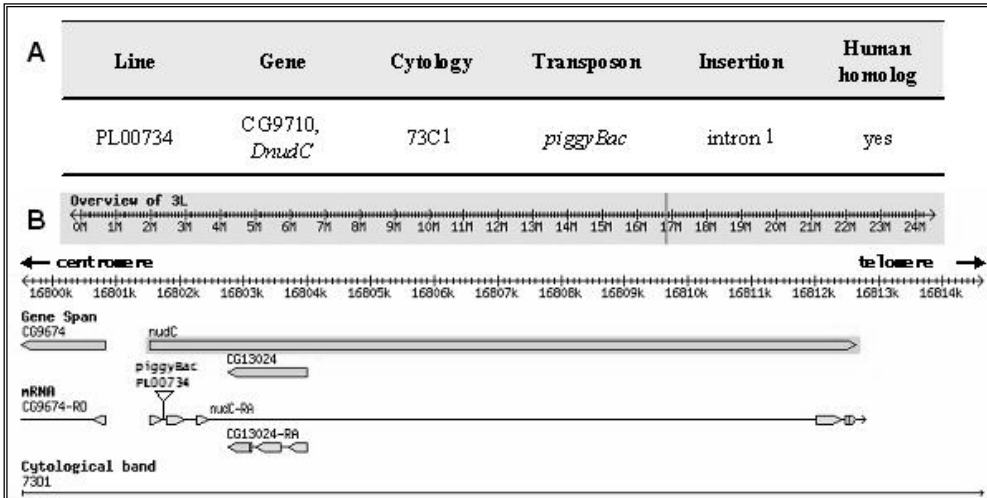
Fig. (2): Localization of the *piggyBac* insertion in the third chromosome lethal stock l (3) PL00734. A) *DnudC* has been mapped to the 73D1 region; B) The piggyback insertion is localized in the first intron, the orientation of the *DnudC* transcription unit is with the 5' end towards the centromer.
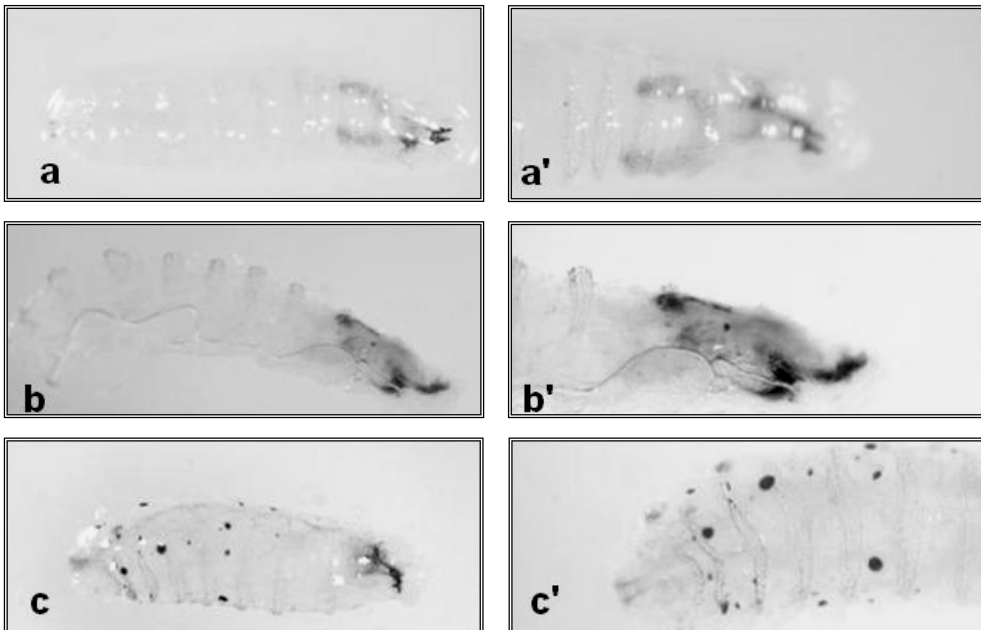


Fig. (3): PL00734 larval-lethal line. Melanotic tumor-like granules in homozygous *nudC* mutant larvae, this over-proliferation phenotype was identified to cause animals to die at second instar larval stage. a and a′) Living larva with necrotic salivary gland tissue b and b′) 2nd instar larva near to die with many necrotic and melanotic tissues in head capsule c and c′) many necrotic lesions on larva cuticle.
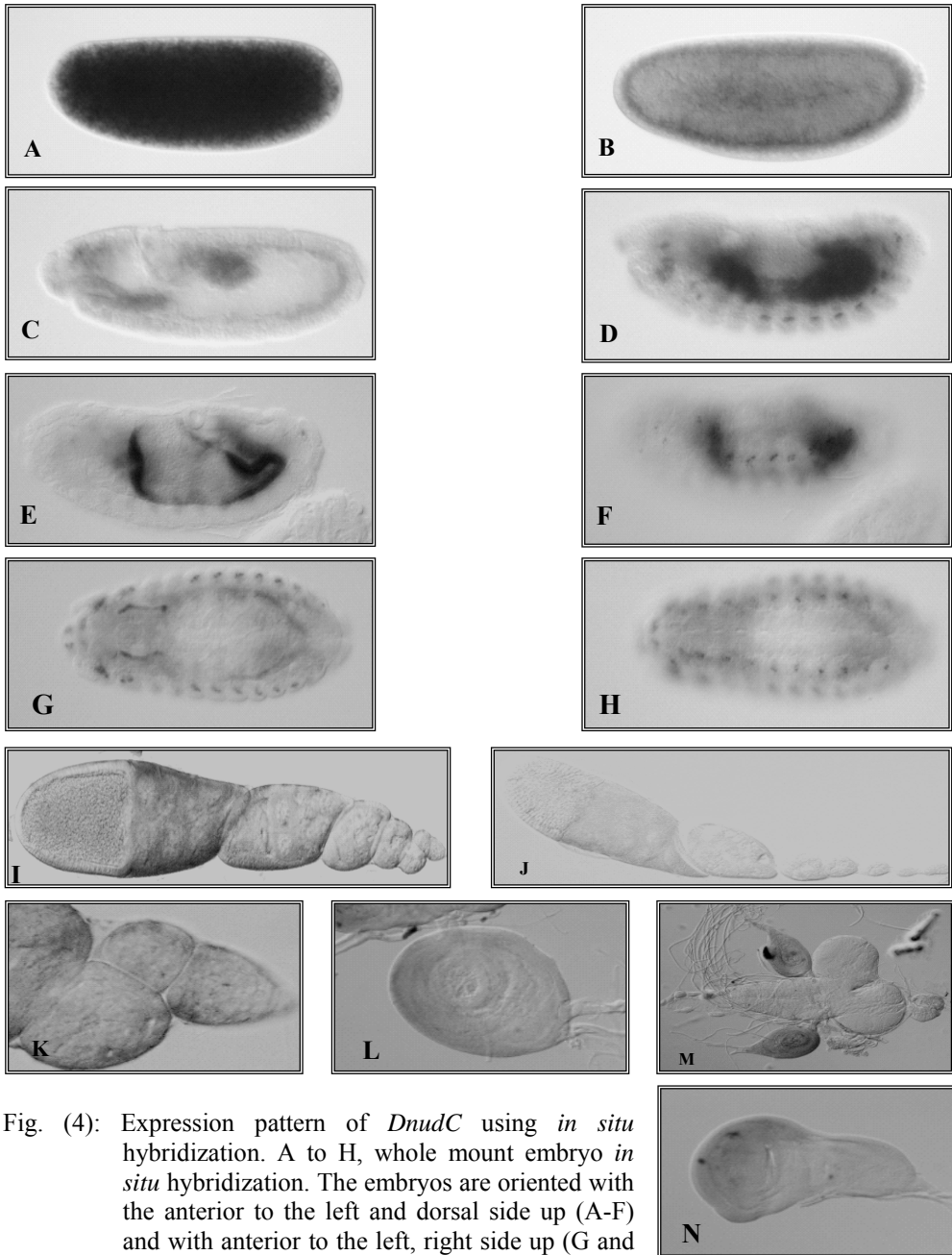
Fig. (4): Expression pattern of *DnudC* using *in situ* hybridization. A to H, whole mount embryo *in situ* hybridization. The embryos are oriented with the anterior to the left and dorsal side up (A-F) and with anterior to the left, right side up (G and H). I to K, ovaries. The ovarioles are oriented with the anterior part to the right. L to M, imaginal discs and brain of third instar larvae. Stages are according to Hartenstein and Campos-Ortega (1985).
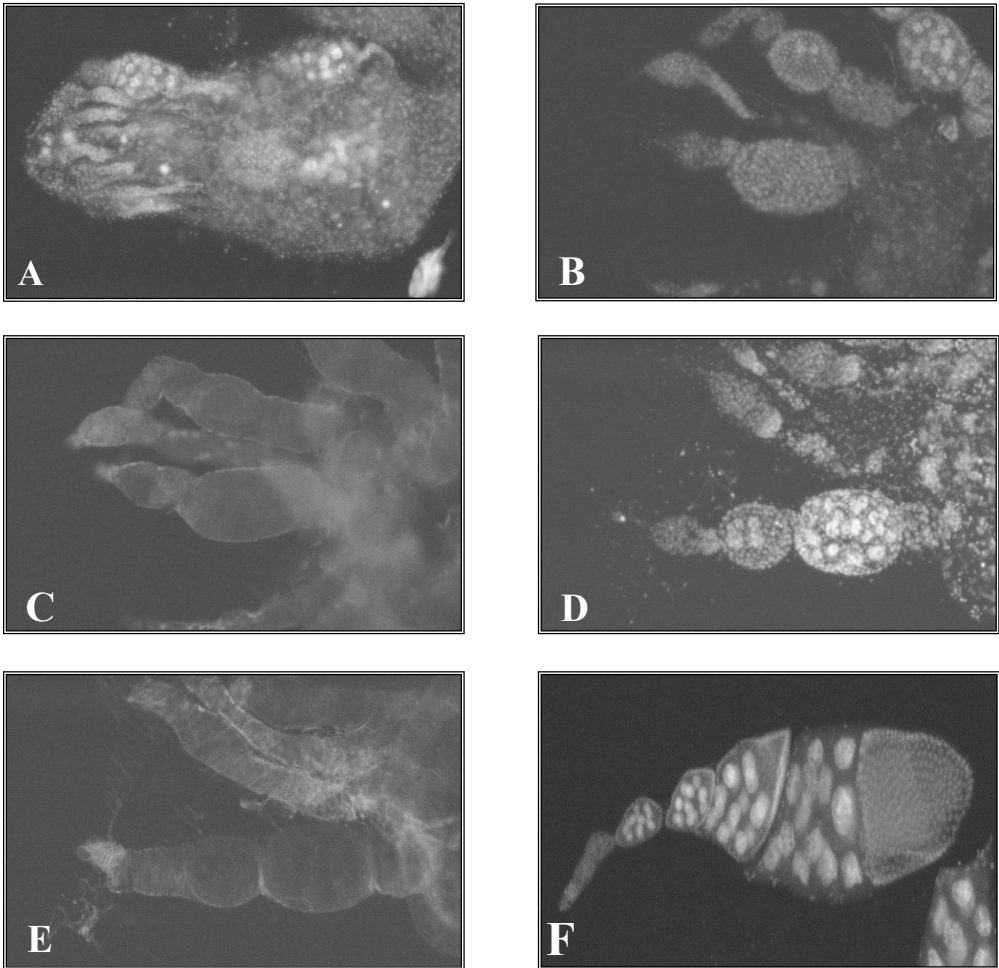
Fig. (5): *DnudC* germline clones. The ovaries are stained with nuclear dye DAPI to visualize DNA (blue), and with rhodamine-coupled phalloidin to reveal actin microtubule architecture (red). (A-E) Egg chambers lacking *nudC* in the germline cells; (A) string-like phenotype related to cell division arrest; (B) likely ovarian tumour phenotype; (D) *ovo*$^{D}$ phenotype; (F) wildtype control egg chambers. The ovarioles are oriented with the anterior part to the left.