

Mutants dissecting development and behaviour in *Drosophila*

Adita Joshi¹, Shanti Chandrashekar¹ and R. P. Sharma^{2,*}

¹Division of Genetics and ²National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi 110 012, India

We have traced in this paper the progress in *Drosophila* genetics research from the 1960s, at the IARI, spearheaded by the visionary insight of M. S. Swaminathan. The work started with the study of indirect effect of radiation and the synergistic interaction of physical and chemical mutagens on chromosomal and genetic changes. This paved the way for the study of single gene mutants in dissecting developmental and behavioural processes. New genes discovered by us have been shown to encode conserved cell signalling molecules controlling developmental and behavioural pathways. With the complete sequencing of the *Drosophila* genome, in the year 2000, mounting evidence for the homology between *Drosophila* and human genes controlling genetic disorders became available. This has led to the fly becoming an indispensable tool for studying human diseases as well as a model to test for drugs and pharmaceuticals against human diseases and complex behavioural processes. For example *wingless* in *Drosophila* belongs to the conserved *Wnt* gene family and aberrant WNT signalling is linked to a range of human diseases, most notably cancer. Inhibition as well as activation of WNT

signalling form the basis of an effective therapy for some cancers as well as several other clinical conditions. Recent experiments have shown that WNTs might also normally participate in self-renewal, proliferation or differentiation of stem cells and altering WNT signalling might be beneficial to the use of stem cells for therapeutic means. Likewise, the *stambha* mutant of *Drosophila* which was discovered for its temperature-dependent paralytic behaviour is the fly homologue of Phospholipase C β . Phospholipase C mediated G protein signalling plays a central role in vital processes controlling epilepsy, vision, taste, and olfaction in animals. Proteins of the G-signalling pathway are of intense research interest since many human diseases involve defects in G-protein signalling pathways. In fact, approximately 50% of the drugs used in clinical medicine target cellular pathways containing G-protein signalling elements. The detailed study of PLC-dependent G protein signalling in *Drosophila* is bound to throw light on the role of G protein-mediated biological functions and on similar genes and their functions in human diseases.

Keywords: Mutants, *Drosophila*, genetics, mutagens, development studies.

Drosophila: An extraordinary model organism for genetics

For over a hundred years, biologists have studied model organisms to examine the mechanisms of inheritance and development. More recently, these processes are being analysed in biochemical and molecular terms, using again the model organisms, to provide insight into gene function. *Drosophila melanogaster*, the fruit fly, has been and continues to be an extraordinarily attractive model organism owing to a combination of its unusually manipulable genetic system, a relatively short life cycle, low cost of maintenance, a huge reservoir of mutants and genetic stocks and biological complexity comparable to that of a mammal. The fly has only four chromosomes and a small genome size of 180 Mb. The sequencing of the *Drosophila* genome completed in 2000 by Celera Genomics and the Berkeley *Drosophila* Genome Project has provided enormous evidence on the value of the fly as a model for human disease, with about 2/3 of human disease genes having a clear cognate in *Drosophila*. *Dro-*

sophila research has already led the way in providing new insights into cancer, neurodegenerative diseases, behaviour, immunity, aging, multigenic inheritance, and development. There are excellent *Drosophila* Web sites, many cross-referenced with one another that provide information on sequence data, images, and other Web resources. FlyBase (<http://flybase.bio.indiana.edu>) at Indiana University provides comprehensive information on the genetics and molecular biology of *Drosophila*. The searchable site supplies genome maps, a browsable image library, lists of stock strains (with an associated ordering mechanism), and contact information for *Drosophila* researchers. The reference database even includes classic texts dating back to the 1920s.

The inception of *Drosophila* genetics research at IARI

During the 1960s and 70s the technique of induced mutagenesis was most effectively utilized in the development of superior cultivars in a large number of crop plants across the world^{1,2}. During the same period radiation was also being advocated for sterilization, disinfection and thus long term storage of food grains and food products. How-

*For correspondence. (e-mail: rpsnrcpb@yahoo.co.in)

ever, the demonstration of the ionization of water and of almost all organic constituents of food and food products by radiations leading to the production of free radicals was considered to have indirect harmful effects of radiations on the consumers. There was therefore a need to dissect in detail the possible harmful effects, if any, of irradiated food. M. S. Swaminathan and his group at the then Botany Division of the Indian Agricultural Research Institute, Delhi were deeply involved in studying the direct and indirect effects of radiation in a large number of plant systems such as wheat, rice, barley, maize, *Vicia faba* and the like. These systems though ideal in certain respects, were not suited to answer some of the most relevant questions relating to somatic and genetic changes caused by ingestion of irradiated food by an organism. It is at this stage that *Drosophila melanogaster* was selected as the organism of choice. Since there was no expertise available at the institute in *Drosophila*, Swaminathan sent Satya Nirula, who had been his Ph D student, to pick up necessary laboratory techniques to rear and work with fruit flies for various aspects of induced mutagenesis from O. S. Reddy at Hyderabad. That is precisely how the work on *Drosophila* genetics was initiated at the IARI. The learning while doing approach under the visionary and able guidance of M. S. Swaminathan motivated one of us (RPS) to expand *Drosophila* work from studying the indirect effects of radiations to understanding the process of induced mutagenesis *per se* and later into the area of developmental and behavioural genetics in association with V. L. Chopra and with the co-author (SC). Looking back in time, introducing and sustaining *Drosophila* genetics research in the agricultural research setup at IARI has been, in our view, a very rewarding and productive experience benefiting both the students and the teaching community alike. Fly research at IARI has grown from strength to strength over four decades following its inception, forever keeping abreast with and applying latest techniques in understanding intricate biological processes.

Direct and indirect effects of chemical and physical mutagens

The question of biosafety of irradiated food was assessed by scoring the incidence of Sex Linked Recessive Lethals (SLRL) among the F₂ families of *Drosophila melanogaster* reared on a basic medium that was irradiated with a sterilizing dose (150,000 rads) of Cobalt-60-gamma-rays. The rate of SLRL mutations in families raised from flies reared on gamma ray irradiated media was ~0.8% as compared to none among those reared on control untreated media³. These studies focused attention of the scientific community and led to a detailed scientific enquiry into the consequences of using radiation for food preservation. Over these years product specific protocols for sterilization of food and food products have been developed and are being extensively used at a commercial scale. Hydroxylamine (HA), 5-Aminouracil

(5 AU) and ICR-70, an acridine half mustard were reported to induce chromosomal rearrangements in *Drosophila*^{4,5}. HA and 5 AU induced a large frequency of centromeric breaks suggesting their preponderance towards affecting GC-rich sequences. ICR-70 had been reported to induce point mutations but not chromosomal aberrations. In their studies⁵, it was possible to demonstrate that ICR-70 does induce complex chromosomal inversions at a concentration where it was shown to induce SLRLs. The alkylating agent ethyl methane sulphonate was shown to induce male recombination⁶. In order to understand the interaction between mutational lesions induced by physical and chemical mutagens administered in sequence, it was demonstrated that post gamma irradiated EMS treatment enhanced the frequency of SLRLs and translocations in *Drosophila* males. The mutation frequency observed in the combined treatments was significantly greater than that obtained by adding the frequencies produced by the two treatments individually⁷⁻⁹.

DNA repair in Hyperkinetic mutant of Drosophila

The *Hyperkinetic* and *Shaker* mutants are both semi-dominant mutations of *D. melanogaster* that have exaggerated shaking of body, legs and scissoring of wings. *Hyperkinetic* (*Hk*), encodes a voltage-gated potassium channel and is short lived because of excessive metabolic rate. To answer the question if high metabolic rate puts the cells under energy stress and thus impairs repair of mutational lesions, the effects of gamma ray-induced mutation rates were studied in *Hk* and *Sh* mutants of *Drosophila*¹⁰. A high rate of dominant lethals and SLRLs but low rate of translocations were observed in *Hk* mutants, thereby supporting the presumption that intrinsic reduced levels of ATP in these mutants are inadequate for repair of mutational lesions. That cells in hyperactive mutants *Hk1* and *Sh5* and their alleles are indeed under energy stress was supported by the observed elevated levels of whole body respiration, mitochondrial respiration, cytochrome-C oxidase activity and mitochondrial ATPase activity.

As a by-product of studying the effect of mutagens and the mechanism of mutation, a large number of mutants became available for study. Several of the mutants had novel and interesting biological effects and became the nucleus of the Developmental Biology and Behavioural Genetics research of the *Drosophila* group.

Developmental mutants determining the body pattern in Drosophila

The development of a complex and organized embryo and later an adult from an apparently homogenous egg had been a central and unsolved biological question for centuries. During early embryonic development, cells in an embryo face two major tasks. First they must be programmed to form specific parts of the body, and second they must realize those fates by altering their shape, position and patterns of gene expres-

sion. Both the determination of cell fate and the corresponding alterations in form occur progressively during development, first under the influence of (pre-zygotic) maternally derived gene products and later under the influence of post-zygotic genes and their products. Deciphering the maternal and zygotic pattern formation genes and their regulatory mechanisms that control the formation, number, and ultimate morphology of body parts is crucial to understanding the dual mysteries of the development and evolution of animal design. Between 1945 and the 1980s three *Drosophila* geneticists, Edward Lewis, Christiane Nusslein-Volhard and Eric Wieschaus, who were later awarded the Nobel prize for Medicine and Physiology in 1995, used the approach of inducing single gene mutations affecting the pattern of the embryo and of the adult to decipher how these genes (both maternally and zygotically acting) and their regulatory networks determine the pattern of the body, beginning from the laying down of the primary axes of the body – the anterior–posterior and the dorsal–ventral. As a consequence of this

pioneering work, *Drosophila melanogaster* emerged as a key model system for elucidating the genetics and molecular biology of animal development.

Wingless: A conserved cell signalling gene in the animal kingdom

The discovery of wingless

Even before *Drosophila* was firmly established as a model for studying genetics of body pattern formation, the IARI fly group was studying and analysing novel and interesting developmentally defective mutants. The discovery of the now well-known *wingless* mutation at the Division of Genetics, Indian Agricultural Research Institute¹¹, the first member of the *wg* gene family (or Wnt gene family as it is now referred to) was among these. The name *wingless* and gene symbol *wg*¹ was proposed for the mutant fly and the gene because of absence of one or both wings (Figure 1).

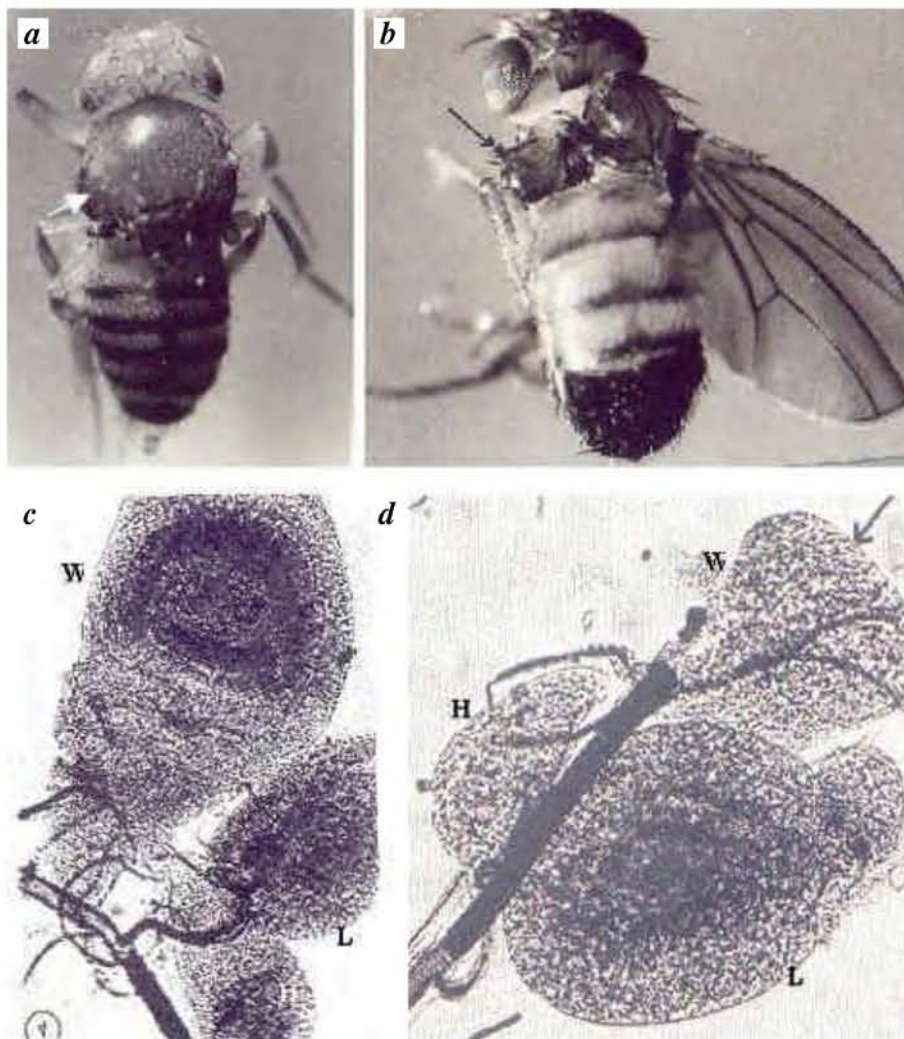


Figure 1. Adult flies and imaginal discs of *wingless*. **a**, Wingless fly with duplications of the notum (arrow). **b**, Single-winged fly with an underdeveloped thorax showing duplications in the notum (arrow). Imaginal discs from a control wild type larva **c**, and from a *wingless* larva **d**. W, Wing, L, leg and H, haltere. Note the absence of presumptive wing blade in wing imaginal disc of *wingless* (arrow).

To understand the biological function of the *wg* gene further, both genetic and developmental studies were carried out¹². Progeny of *wg*¹ flies usually had wingless, one winged and two winged flies in a 2:2:1 ratio. On crossing males and females of the one winged and two winged classes, the progeny continued to segregate into the three phenotypes. Efforts towards obtaining a homogenous population of *wg*¹ flies were not productive, though the proportion of *wg*¹ flies increased during the consecutive cycles of crossing and selection. The gene was mapped by meiotic recombination to ~18cM to the left of *black* (2–48.5cM) placing *wg* at ~30cM which subsequently has been corrected to 21.9.

Developmental studies on wg mutant flies

Besides showing absence of wings (Figure 1 a), *wg*¹ flies showed absence of halteres and flies with all combinations of two wings, one wing and no wings with and without one or both halteres were observed (Figure 1 b). The other significant phenotype was a duplication of the notum, partial or complete absence of mesothoracic bristles, an absent or deformed scutellum, irregular arrangement of hairs, hemithoracic or thoraxless flies. All these abnormalities pointed very strongly to the possibility that *wg* gene acted at a very early stage of development to serve some fundamental role. The wing and notum in *Drosophila* develop and differentiate from groups of ectodermal cells called the imaginal discs that are present in the internal body cavity of larvae. A fully developed wing imaginal disc has an anterior region which differentiates into thorax and a posterior part which forms the wing (Figure 1 c). The wing discs dissected from *wg*¹ third instar larvae were found to lack the posterior presumptive wing region with an intact anterior part that gives rise to the notum (Figure 1 d). This observation connected wing disc development in *wg* flies with that of the observed morphological wingless condition. With this information as the base and also the various instances of mesothoracic abnormalities, it was hypothesized that the *wg* mutation prevents the wing blade development in mesothoracic discs resulting in a duplication of the notum much akin to the differentiation of duplicated nota observed when anterior wing disc fragments were cultured. To explain the various phenotypes of thoraxless and partial notum phenotypes it was further hypothesized that the *wg* mutation affected wing and thorax development at multiple steps during wing specification in a compartment-specific manner. It was proposed thus that *wg*⁺ function was necessary and indispensable for normal wing and haltere disc development and proper assignments of their morphogenetic fates during *Drosophila* development.

wg action – the cell death hypothesis. Cell death vs homeosis

Several new alleles of *wg* were isolated in the hope of finding a fully penetrant *wg* allele or a temperature sensitive allele

to study the stage of development at which the *wg* gene exerted its function. However, none of the nine viable alleles isolated were fully penetrant or temperature sensitive¹³. To explain the absence of the presumptive posterior wing cells of the wing disc it was proposed that the *wg* gene product induces cell death during wing imaginal disc development. Death of the presumptive wing region leads to ‘wound healing’ and a respecification of cell fates during disc regeneration. This explanation drew its strength from the studies that showed mirror image duplication of thorax when the posterior part of the mesothoracic (wing) disc is surgically cut or eliminated due to cell death (as in discs of *vg*, *Sd* and *wingless*)¹⁴. To confirm the reduction in cell number due to apoptotic events, staining of dead cells was done using two different staining dyes acridine orange and neutral red. Both the stains could locate larger zones of dead cells in mutant and wild type discs¹³ and considerable increase in the number of dead cells in the mutant discs was recorded. It appeared that *wg* either itself was inducing cell death or was somehow altering the process of programmed cell death. *wg* probably triggered its action initially when the wing notum compartment is laid down and could induce the spread of cell death over entire region. The cells escaping *wg*¹ action would yield mirror images or would develop normally whereas those undergoing degeneration or death would give distinct morphological variants. Bryant¹⁵ also ascribed position-specific developmental capacities to various cells in a developing imaginal disc and demonstrated that cells at a ‘lower’ level of developmental capacity can only duplicate whereas those at the higher level can regenerate the missing parts. Duplication of notum, parts of scutum and notopleural structures in *wingless* conformed well with the model wherein they could only produce mirror images in the absence of the presumptive wing which being at a higher level could have generated the normal notum.

Apart from the cell death model to explain the *wg* phenotype Babu¹⁶, and Morata and Lawrence¹⁷, proposed that *wg* was a new homeotic gene in *Drosophila* which could cause wing to notum transformation. The IARI group nevertheless suggested that *wg* was in all likelihood not a homeotic gene because in *wg* flies the normal notum showed several reduced phenotypes whereas homeotic genes are not known to affect the compartment that is not transformed.

Cell patterns, segment polarity and morphogens – the role of wingless signalling

The question of the biological function of *wingless* was elegantly answered in 1980, by Nusslein Volhard and Eric Wieschaus¹⁸, seven years after the discovery of *wg*.

In the *Drosophila* embryo, visible patterning of the segments, in particular the bands of cuticular denticle belts results from periodic variation in morphogen levels. These patterns provided the classic experimental screen for developmental patterning mutants¹⁸. They isolated a lethal allele of *wg* in

a screen for zygotic mutants that affect larval cuticle pattern and reported that *wingless* is a segment polarity gene, and its pattern of expression is responsible for the banding of cuticular denticles and for determining segment polarity. Early polarization of the fly embryo directly involves both mRNA localization and diffusion gradients of gene-regulating proteins, because the embryo does not subdivide into cells until about 3.5 h after fertilization. After cellularization, cell membrane barriers block direct passage of macromolecules from nucleus to nucleus, and spatial information must be defined by morphogen gradients consisting of extracellular macromolecules, usually proteins. The extracellular morphogens must be linked to gene regulation by intracellular signalling pathways. These morphogen signals fall into two classes: (i) *Direct cell to cell interactions or short range morphogen*, i.e. by interaction of a signalling protein on the cell surface of the upstream cell with a receptor on the downstream cell; signals of this type may be confined to a boundary layer. (ii) *Diffusible factors* released by the upstream cell and activating a receptor on the downstream cell. Such signals may penetrate many cells deep into the target tissue and are of long range (on a cellular scale). However real distances are rarely more than a fraction of a millimeter. Homeotic gene products activate expression of a short range signalling protein, which establishes an organizer region. The organizer region then secretes diffusible signalling proteins (*Decapentaplegic*, *dpp* and *wingless*, *wg*) which form gradients controlling developmental fate of cells along each axis. The protooncogene coded by *int* gene (involved in integration of mouse mammary tumor virus in mammary tumours) became the archetype for this class of signalling factor. The *Drosophila wingless* gene was recognized as a homolog of *int* in 1987 giving rise to the designation Wnt as the concatenation of *Wg* and *int*^{19,20}.

In *Drosophila* the cuticular denticles form on cells at the anterior edge of the segments. The segment polarity gene *engrailed* is expressed at the posterior boundary of the previous segment and activates expression of the short range morphogen, hedgehog (Hh). Hedgehog induces boundary cells on its anterior side to express the diffusible signalling factor wingless (*Wg*). The same interactions are repeated at the next segment boundary to the right (posterior). Hedgehog promotes denticle, whereas wingless signalling specifies smooth cuticle²¹ (Figure 2). *Wg* mutants are uniformly covered in denticles, as are the mutants *porcupine*, *dishevelled* (*dsh*), *armadillo* (*arm*) and *pangolin* while *Zeste white3* (*zw3*) mutants show the opposite phenotype, a naked cuticle.

Segment polarity to cancer: The *wg-int-1* connection

The identification of *wingless* as the *Drosophila* homolog of a vertebrate oncogene *int-1* that resulted in mammary tumours in mouse followed by insertion of Mouse Mammary Tumour Virus (MMTV) was an important milestone that led to the

discovery of *wingless* related genes in other organisms. Following the connection between *wg*, *int* and cancer, over 100 Wnts are now catalogued in other organisms from *C. elegans* to humans (The *Wnt* Homepage <http://www.stanford.edu/~rnusse/>).

The striking homology between *int-1* and *wg* went hand in hand with the observed facts that both of them are active where a developing tissue is involved – tumorigenesis in mouse mammary gland occurs only after cycles of growth and development followed by regression after pregnancy. In the fly, growth followed by proper differentiation of the epidermis is mediated by the *wg* protein through cell–cell communication. This finding presented a strong reasoning towards the cell signalling role of a particular *Wnt* gene family member in different organisms in varied development phenomena. The *Wnt* gene family, the members of which secrete glycoproteins characterized by a signal sequence and a cysteine-rich sequence of 23 invariant Cys residues thus made a foray into the scientific world. They act via cell signalling and are intrinsic to the most fundamental and intriguing phenomenon that is – Development. *Wnt* genes are involved in processes as diverse as pattern formation, cell determination and differentiation, tissue induction, cell fate determination, axis specification, etc. (Table 1). In *Drosophila* the *wg/Wnt* signalling cascade is identified from mutant phenotypes of several other genes like *porcupine* (*porc*); *dishevelled* (*dsh*); *armadillo* (*arm*) and *pangolin* (*pan*). Loss-of-function mutants in all of these genes show phenotypes identical to that of *wg*. This is consistent with defects in *wg* signalling. Conversely, a loss-of-function mutation in *zeste-white* has a phenotype opposite to that of *wg*, *porc*, *dsh*, *arm* and *pan*. Double mutants for different combinations of these genes have been used to arrange the genes in order of their biological effects (Figure 3). The vertebrate counterparts of these genes are also known indicating conserved signalling during a variety of developmental events and pathways throughout the animal kingdom (Table 1, Figure 3).

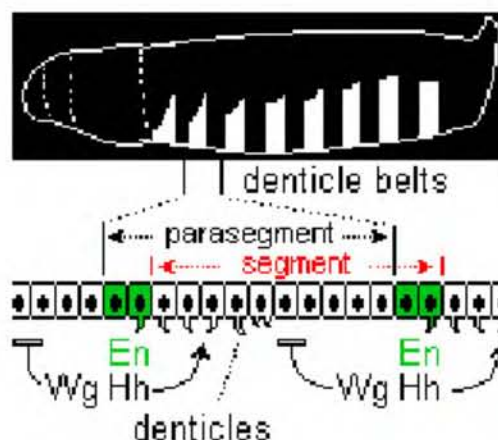


Figure 2. Wingless expression and segmentation pattern in *Drosophila* embryos.

Table 1. Involvement of *Wnt* genes in animal development

Developmental event/program	Organism
Pattern formation	<i>Drosophila</i> (epidermis, brain), Mouse (brain)
Mammary carcinogenesis	Mouse
Cellular differentiation and determination	<i>Drosophila</i> (neuroblasts)
Tissue induction	<i>Drosophila</i> , Mouse, <i>C. elegans</i> , Humans
Cell fate determination	<i>Drosophila</i> , <i>C. elegans</i>
Generation of cell diversity	<i>Drosophila</i> , <i>C. elegans</i>
Cellular proliferation	<i>Drosophila</i>
Imaginal disc development	<i>Drosophila</i>
Axis specification	Xenopus, Mouse
Limb formation	Xenopus, <i>Drosophila</i>
Segment polarity	<i>Drosophila</i>

expression. Thus the exact role these genes play in the wild type fly is a mystery till date.

Altered *Wnt* signals and human disease

The human genome has nineteen *Wnt* genes. There exists enormous diversity in medical conditions ranging from schizophrenia and leukaemia to limb duplication (tetra amelia) that involve aberrations in the *Wnt* signalling pathways in humans (Table 3). *Wnt* signalling can be broadly divided into two streams depending on the recruitment of beta catenin.

Beta-catenin dependent pathway

Beta-catenin is the key mediator of the *Wnt* signal wherein the nuclear functions of beta catenin is triggered to influence gene expression controlling cell proliferation and survival. Frizzled and LRP's (LDL-related proteins) are the known receptors of this pathway. In cells not exposed to the *Wnt* signal, beta-catenin levels are kept low through interactions with the protein kinase zw3/GSK-3, CK1a, APC and Axin. Beta-catenin is degraded, after phosphorylation by GSK-3 and CK1 alpha. *Wnt* signalling initially leads to a complex (between Dsh, GBP/Frat1, Axin and Zw3/GSK) that does not phosphorylate beta-catenin anymore and the stabilized beta-catenin then enters the nucleus to interact with T-cell-specific transcription factor (TCF). Beta-catenin can convert TCF into a transcriptional activator of the same genes that are repressed by TCF alone. Activating mutations in the human beta-catenin gene have been found in human colon cancer and melanomas. These mutations alter specific beta-catenin residues important for GSK3 phosphorylation and stability.

Beta-catenin independent pathways. There are a few other cascades which influence changes related to cell movement and polarity, for example the *Wnt* calcium pathway which also has some functional overlap with *Drosophila* planar polarity pathway.

Wnt signalling in humans invites great interest as mutations and deviations associated with the components or regulators of this pathway are implicated in several clinical conditions. Very often beta-catenin is the molecule central to these conditions as it exists at the junction wherein several other pathways like notch, tgf-beta and *Wnt* intersect each other. There are dramatic consequences of loss of *Wnts* or other signalling component's function. The autosomal recessive disorder tetra amelia (loss of all four limbs) is due to a nonsense mutation in *Wnt3* (ref. 27). Loss of function mutations in *FZ4* and *LRP5* cause familial exudative vitreoretinopathy which is an inherited disorder of the retinal vasculature leading to blindness. Increased bone density in jaw and palate is reported to occur due to a single amino acid substitution in *LRP5* (refs 28, 29). The same molecule is also involved in instances of decreased bone mass wherein a loss

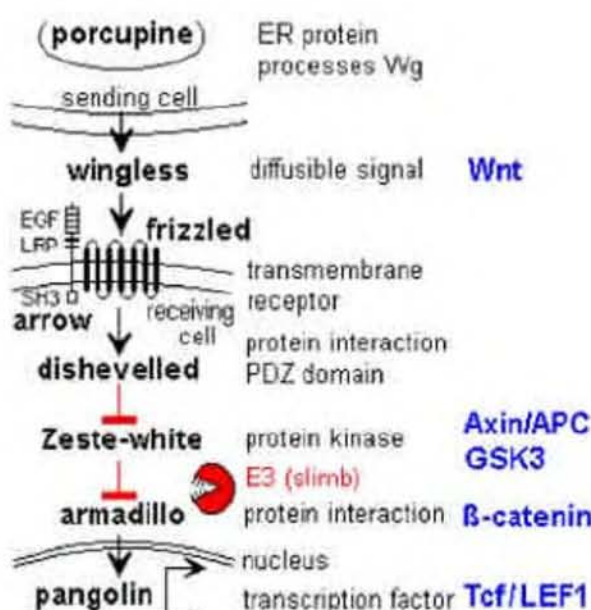


Figure 3. The canonical *wg-frizzled* signalling pathway in *Drosophila*. Vertebrate homologs of wingless are shown in the extreme right column.

The fly has seven *Wnt* genes

In *Drosophila* seven *Wnt* homologues have been reported (Table 2) of which *wg* remains the most extensively studied. *DWnt2*, *DWnt3*, *DWnt4*²²⁻²⁴ and *DWnt6*, *DWnt8* and *DWnt10* were identified recently with the sequencing of *Drosophila* genome²⁵.

So far loss of function mutations has been obtained only for *DWnt2*, demonstrating its requirement for the development of male reproductive tract²⁶. There exists only a scanty knowledge about the functional diversity among other *Wnts* as none of them was identified in mutant screens rather their existence in the fly was known by sequence homology. Current understanding of these genes has mainly come from patterns of expression or phenotypes caused by over

Table 2. *Wnt* genes in *Drosophila*

Gene	Location	Phenotype	Vertebrate ortholog
<i>Wingless</i> , CG4889 in Flybase	27F	Segment polarity	<i>Wnt1</i>
<i>DWnt2</i> , CG1916 in Flybase	45E	Pigment cells gonads; adult muscle trachea (with <i>wg</i>)	<i>Wnt7</i>
<i>DWnt3/5</i> , CG6407 in Flybase	17B	Axon guidance (through <i>Derailed</i>)	<i>Wnt5</i>
<i>DWnt4</i> , CG4698 in Flybase	27F	Cell movement in ovary	<i>Wnt9</i>
<i>DWnt6</i> , CG4969 in Flybase	27F	Mutants not available	<i>Wnt6</i>
<i>DWnt8</i> , CG8458 in Flybase	87E	Mutants not available	No ortholog
<i>DWnt10</i> , CG4971 in Flybase	27F	Mutants not available	<i>Wnt10</i>

Table 3. *Wnt* and β -catenin pathway genes involved in human diseases

Gene	Condition/disease	Mutation or activity
<i>Wnt1</i>	Schizophrenia	Elevated
<i>Wnt3</i>	Tetra amelia	LOF
<i>Wnt4</i>	Intersex	GOF
<i>Wnt4</i>	Kidney damage	Elevated
<i>Wnt4</i>	Polycystic kidney disease	Variable
<i>Wnt5a</i>	Leukaemia	LOF: reduced
<i>Wnt5a</i>	Metastasis	Elevated
<i>sFRP3</i>	Osteoarthritis	SNP: reduced
<i>FZA</i>	FEVR	LOF
<i>LRP5</i>	FEVR low bone mass	LOF
<i>LRP5</i>	High bone mass	GOF
<i>DSH/DVL</i>	Lung cancer	Elevated
<i>APC</i>	Cancer	LOF
<i>AXIN</i>	Cancer	LOF
<i>AXIN2</i>	Cancer, tooth agenesis	LOF
β -catenin	Cancer	GOF
β -catenin	Aggressive fibromatosis	Elevated
β -catenin	Pulmonary fibrosis	Elevated

LOF, Loss of function; GOF, Gain of function; SNP, Single nucleotide polymorphism; FEVR, Familial exudative vitreoretinopathy; LRP-LDL, Related protein; FRP, Frizzled related protein.

of function mutation occurs in *LRP5* (ref. 30). Osteoporosis pseudoglioma syndrome (OPPG) is another outcome of *LRP* mutations³⁰. Oligodontia, a condition in which multiple permanent teeth are missing is due to a nonsense mutation in *Axin2*, an intracellular component of *Wnt* pathway³¹. Individuals with *Axin2* defects are also predisposed to colon cancer³¹. Familial adenomatous polyposis (FAP) is a disease leading to formation of thousands of polyps in rectum and colon. This is a result of truncated APC product which promotes aberrant activation of the *Wnt* pathway^{32,33}. Axin is reported to be involved in several other carcinomas too. Thus, it becomes evident that any perturbation in normal beta catenin regulation by *Wnts* is a triggering event leading to cancer. With this information several therapeutic approaches involving the use of inhibitors of Wnt- β catenin signaling such as small molecules that block interaction of tcf with β catenin, si RNA's, and antibodies against *Wnts*, are being tested.

Apang – a dorsoventral patterning mutant. While evaluating temperature-sensitive lethal mutations on the second

chromosome of *Drosophila*, adult flies of one of the 'lethals' had defective tarsal segments on all six legs at 28°C but had normal legs and tarsi at 19°C. On closer examination the tarsal segments showed expansion of ventral structures at the expense of dorsal structures – a 'ventralization' of the leg³⁴ (Figure 4). The mutation causing this phenotype was mapped to 6.7 cM on chromosome 2L and named *apang*. The mutation was later on shown to cause a similar ventralization of the embryonic cuticle and of wing structures (Chandrashekar, unpublished) (Figure 4). It was found to cause widespread cell death in the dorsal regions of leg discs. It was lethal in double mutant combination with *decapentaplegic*, a gene which is shown to be involved in determination of ventral structures in the embryo and in all the imaginal discs. *Apang* was also necessary for nurse cell viability and ovarian development, but was primarily seen to control dorso-ventral polarity in the embryonic and adult structures.

Spiny leg-bristle orientation reversal. Four single gene mutants – *prickled* (*pk*), *spiny leg* (*sple*), *frizzled* (*fz*) and *inturned* (*in*) known to affect the polarity of cuticular hairs (bristles) were reported in *Drosophila*. A temperature-sensitive autosomal (second chromosome) recessive mutation possessing highly deformed legs with severe segmentation abnormalities and a unique phenotype of reversed bristle orientation on the leg at 28°C was isolated from a screen of F₃ EMS mutagenized lines. Legs showed condensed and fused femur, tibial and tarsal segments, incomplete intersegmental joints, irregularly oriented leg bristles and very often an increase in bristles (Figure 5). The mutant phenotypes were exaggerated at 19°C as a consequence of which the legs acquired a stubby and spiny appearance³⁵ and was named *sple bristle orientation reversal*^{ts} (*bor*^{ts}) (subsequently redesignated a novel allele of *pk* as *pk*^{*bor*^{ts}}). Reversal of bristles and its associated leg phenotypes in *bor*^{ts} were altogether different from those of the earlier known four mutants. No significant changes were observed in the area of mutant third instar larval leg disks or in their compartmentation pattern as revealed by aldehyde oxidase histochemical assays. The leg deformities were expressed in ectopically developed cephalic legs of the homeotic mutants *spineless aristapedia* and *Antennapedia* demonstrating that the bristle and joint defects

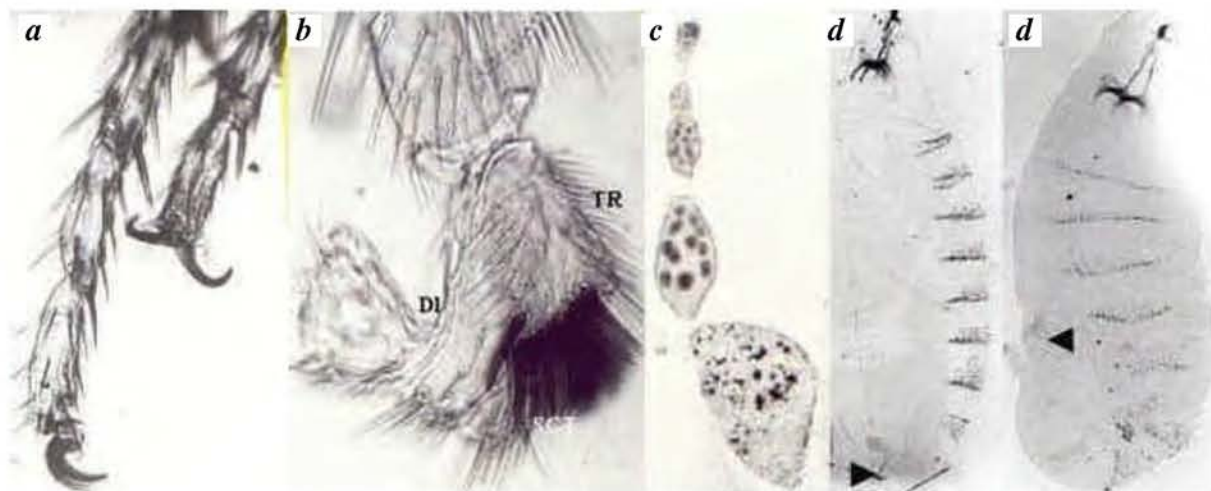


Figure 4. *Apang* – a dorsoventral patterning mutant. *a*, Tarsal segments of a wild type fly; *b*, A ventralized *apang* male prothoracic leg. Note the duplicated Sex Comb teeth (SCT), transverse rows of bristles (TR) – both ventral structures – and reduced dorsal cuticle (DI); *c*, A single ovarian follicle showing denaturing nurse cells at stage 8–9; *d*, A mature wild type embryo showing the ventrally placed filzkörper (arrow head). Embryo is oriented anterior to the top and ventral to the right; *e*, A ventralized *apang* embryo showing the filzkörper (arrowhead) at the dorsal surface.

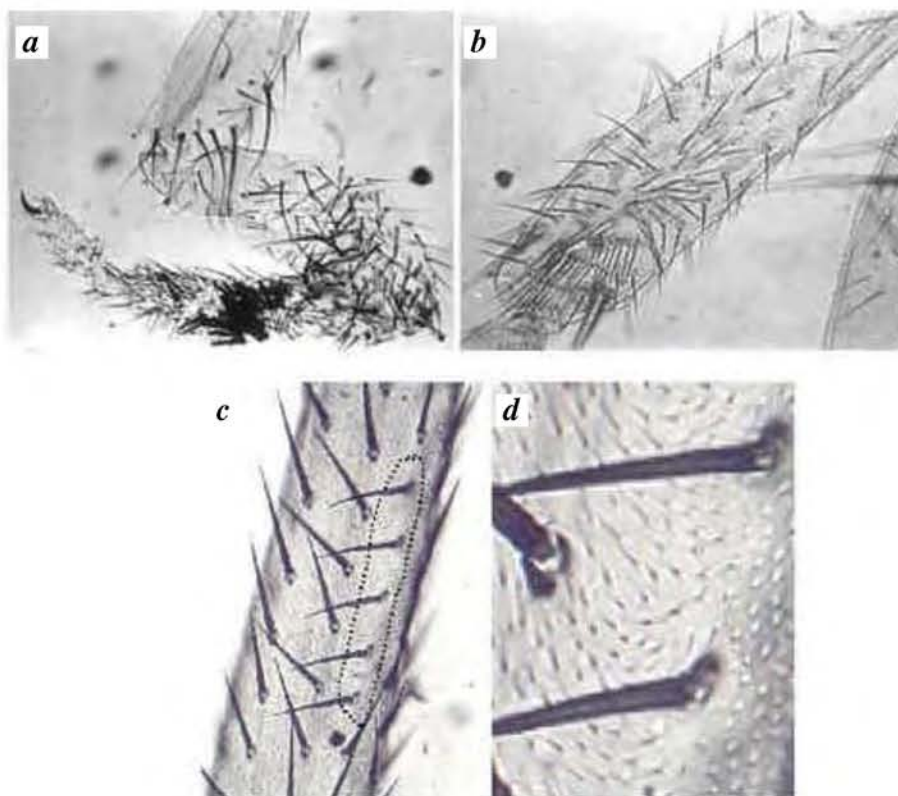


Figure 5. Bristle orientation reversal in *sple bor ts*. *a*, The low temperature phenotype showing bristles in disarray; *b*, The metatarsal segment showing orientation reversal of bristles; *c*, A somatic clone of *pk^{bor ts}* showing reversal of bristles (induced in a *pk^{bor ts}/+* background); *d*, Enlarged view showing the gradual change in the orientation of hairs surrounding the bristle.

were leg specific. Somatic clones of *pk^{bor ts}* cells were studied in a wild type background in adult legs to test if the mutation was autonomous in its effect and whether it affected cell multiplication and patterning (thereby altering clone

shape and size). Clones of *yellow* marked cells in a wild type background were used as control. *bor* clones showed autonomy of expression of reversal of bristle orientation and did not affect the orientation of any neighbouring

non-*bor* cells (Figure 5). Control *yellow* clones in a *bor* background were broader in width and irregular in shape compared to similar clones induced at the same stage of development in *bor+* individuals (Figure 5). The polarity reversal in the mitotic clones as revealed by the orientation of bristles and trichomes was found to be gradual (in small increments) like in a whorl and not a sudden single step reversal of bristle orientation. The existing models in *Drosophila* explaining planar polarity of cells within segments were found inadequate to explain the various phenotypes of *pk^{bor ts}* and a new cell polarity model (Sharma and Chitnis, unpublished) with the following tenets was put forth.

(i) Cell polarity is determined by the distribution of various cell surface (membrane bound) components. (ii) Each cell surface has a unique combination of cell surface components. (iii) Each surface has a given affinity to another cell surface. The highest cell–cell affinities determine the most stable cell contacts. (iv) Cells have inherent capacities to reorient themselves (without shifting their physical position) in order to achieve the highest intercellular affinity. (v) The initial imprinting of polarities is determined by early embryonic gradients but once specified it is autonomous and maintained during cell division and cell movement.

Maternally acting genes controlling the embryonic body pattern

With the isolation and study of *apang* and the growing excitement of the discovery of pattern forming genes in *Drosophila*, there was a natural interest in the study of genes affecting embryonic pattern formation. Female sterile and maternally acting embryonic lethal mutations served as useful entry points to study genes whose wild type products are synthesized before fertilization but are utilized during early embryogenesis. Although most of the gene determination and body pattern had been discovered, it became apparent that the genome had not truly been ‘saturated’ for all possible genes that might regulate this process. Garcia-Bellido and Moscoso del Prado³⁶ by using a cytogenetic approach to compare the contribution of the maternal genome in embryonic development showed that there were several stretches of the genome that were unable to support normal embryonic development when the dosage of those regions was haploid (instead of the usual diploid). The regions were therefore maternally haplo-insufficient for normal embryonic development. Garcia-Bellido *et al.*³⁷ assessed the maternal contribution of the genome by comparing survival of embryos derived from hemizygous (Df/+) mothers or fathers mated to wild type parents. However, no distinction was made between eggs that died before fertilization and embryos that died after fertilization. It is important to distinguish between pre- and post-fertilization egg mortality because high pre-fertilization mortality in a Df/+ female X +/+ male mating does not necessarily imply maternal haplo-insufficiency.

We assessed the maternal and paternal hemizygosity effects of several deficiencies spanning chromosome 2 on both pre and post fertilization embryonic mortality in crosses and discovered that there was significant post-fertilization embryonic mortality in Df/+ X +/+ crosses where the Df lines were Df(2L) E55 37D2-38A1, Df(2L)al 21 B8.C1-21 C8.D1 and Df(2L)S3 21D2-22A1. This suggested that the genomic regions represented by the deficiencies were insufficient in a single maternal dose for normal embryonic survival. A literature search for reports of maternally active embryonic lethal mutations in these haplo-insufficient regions revealed that no such genes had been reported in any of the three regions. To confirm if maternal haploinsufficiency of the selected genomic regions was indeed due to the haploid dosage of maternally acting loci the *Drosophila* genetics group in 1985 embarked on a search for maternally acting embryonic lethal mutations in these genomic regions of chromosome arm 2L. A new and efficient screening system utilizing a newly synthesized second chromosome balancer SM5 carrying a Dominant Temperature Sensitive Lethal^{38,39} was employed for this purpose. Around 12,000 mutated chromosomes were screened for putative female sterile maternally acting lethal (*fs mel*) loci as a result of which 17 alleles of 9 *mel* genes were discovered⁴⁰⁻⁴². Seven of the 9 *mel* loci were in the 37D2-38A1 region (Table 4) and one mutant each was located in the 21 B8.C1-21 C8.D1 and 21D2-22A1 regions respectively. The *mel* mutants affected early mitotic nuclear divisions, pole cell formation, gastrulation and the number and pattern of segments in the first instar larva. These results also demonstrated several things. First, it confirmed the assumption of Garcia-Bellido³⁷ that maternal haplo-insufficiency of genomic regions is indeed due to maternal hemizygosity of *mel* loci. Second, that several maternally acting loci had escaped detection in the large mutation screens that had been conducted and that systematic analysis of similar maternally haplo-insufficient regions would yield more information on new *mel* loci and third, that useful biological material to begin new investigations was available.

Behavioural genetics

During the 1970s, *Drosophila* was accepted as a very good model system for studying the genetic regulation of neural structure and function, and the relationship of such regulation to behaviour. The study of mutations and the genetic manipulation of behaviour in *Drosophila* was pioneered by Seymour Benzer⁴³. *Drosophila* mutants exhibiting a variety of behavioural alterations such as rapid paralysis, abnormal response to light, abnormal sexual behaviour and locomotory behaviour became valuable for investigating the *in vivo* roles of gene products in neuronal development, neural function, synaptic transmission and signalling pathways⁴⁴⁻⁴⁶. In the 1980s the *Drosophila* laboratory at IARI began genetic investigations of several of such behavioural mutations and discovered several new genes.

Table 4. Maternal effect embryonic lethal (*mel*) mutants identified in the 37D2-38A1 cytological region

Complementation group (alleles)	External egg structure	Embryonic phenotype at arrest
<i>mel1</i> (1)	Short stubby chorionic appendages	32-64 cleavage nuclei
<i>me 2</i> (3)	Normal	<i>mel2</i> ³ -syncytial blastoderm, <i>mel2</i> ¹ , <i>mel2</i> ² -head skeleton deleted
<i>mel3</i> (3)	Short egg, translucent chorion, fused, stubby chorionic appendages	<i>mel3</i> ¹ -8-16 nuclei, <i>mel32</i> , <i>mel33</i> -posterior abdominal segments deleted
<i>mel4</i> (2)	Normal	Cephalopharyngeal skeleton absent, abdominal segments narrow in width
<i>mel5</i> (2)	Normal	Variable between syncytial and cellular blastoderm
<i>mel6</i> (1)	Normal	Club-shaped head, weakly formed abdominal segments
<i>mel7</i> (1)	Normal	Twisted embryonic cuticle, reduced ventral cuticle bands

Light-induced homosexual behaviour

Sexual activity in *Drosophila* males, like in any other complex animal, is controlled by the function of a large number of genes. During a screen for light-induced behavioural mutants an interesting light-dependent homosexual mutant was discovered⁴⁷. The mutant was associated with an X and third chromosome translocation and was recessive in nature. While sexual behaviour in wild type males was not influenced by light, the mutant males showed homosexual behaviour under light, forming chains of males and attempted pseudo-copulatory behaviour. Yellow light (wavelength 575 nm) induced maximum pseudocopulation among males whereas red light instantaneously terminated the sexual activity. The flies of this mutant line were otherwise slow in locomotion and the abnormal sexual behaviour was in all likelihood a subset of other complex behavioural abnormalities.

Temperature sensitive paralytic mutants

Ever since the discovery of the temperature sensitive (TS) paralytic mutant *paralysed*¹⁵ on the X chromosome of *Drosophila melanogaster*⁴⁴, several similar single gene mutants with similar phenotypes were discovered, predominantly on the X chromosome because of the ease with which X-linked mutations could be screened. The autosomes, however, were not visited very frequently and several *ts* paralytic mutants were waiting to be discovered. In 1983 a systematic screen for reversible recessive adult *ts* paralytic mutants on the second chromosome was conducted and mutations in three novel genes (all mapping to chromosome 2R) and subsequently named *stambh A*, *stambh B* and *stambh C* were discovered and described⁴⁸. These loci were mapped on chromosome 2 at genetic positions of 56.7, 97.5 and 59.3 cM respectively. Adult flies and larvae homozygous for any of the three mutant loci paralysed within minutes of exposure to a non-permissive temperature of 35°C and above and revert to normal mobility on transferring back to permissive temperature of 23°C. Of the three paralytic mutants *stmA* showed temperature-dependent

developmental abnormalities and was selected for detailed investigations due to its likely influence on a variety of developmental events.

stambh A. The *stambh A* (*stmA*) *ts* paralytic mutant showed heat-induced embryonic lethality besides exhibiting paralysis at non-permissive temperature of 35°C. Adult flies also showed a reduced sensitivity to the lethal effects of a sodium channel specific neurotoxin veratridine^{48,49}, suggesting that *stmA* possibly affected some component of voltage-gated sodium channels or the nerve membrane. Subsequently several new alleles of *stambh A* were isolated, some of which were recessive lethal and died as embryos with a hypertrophic embryonic nervous system and loss of embryonic cuticle⁵⁰. This phenotype of neural hypertrophy where the nervous system proliferates at the expense of the epidermis is typical of mutations in a group of genes called the 'neurogenic' group of genes the products of which have widespread roles in cell-cell communication. It was demonstrated that *ts* paralysis is due to a gain of function mutation and embryonic lethality due to a loss of function. Twenty two *stmA* alleles⁵¹ when deployed in inter-allelic complementation studies demonstrated that *stmA* is a complex gene and has more than one functional domain. It was also demonstrated that *stmA* is maternally required for cuticle development and is necessary for female germline viability⁵¹. The cytogenetic position of *stmA* was determined to be 44D1-2 (ref. 52). A genetically engineered enhancer-trap P transposable element located in 44D4-5 was locally mobilized into *stmA* to create transposon tagged mutant alleles (Chandrashekar, unpublished). Genomic DNA flanking the P insertion sites have been recovered and have been found homologous to the *cmp44E* gene (discussed below).

stambh A codes for an evolutionarily conserved membrane bound phospholipase C in the G protein signalling pathway

The *stmA* gene was first cloned by Faulkner *et al.*⁵⁴ and shown to encode a conserved membrane-bound protein (*cmp44E*) with several transmembrane sequences and an

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