Wingless to Wnt: discovery of conserved cell signalling gene family in the animal kingdom

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Drosophila, a model system for genetic dissection of development

Genetic dissection of the process of development in animal systems has revealed that there are three kinds of genes, i.e. maternal effect genes, segmentation genes and homeotic genes that govern the basic structure of a multicellular organism. The interplay of these genes which can be sequential, superimposed and interactive and separated in time and space ensures that cells take well-defined, stepwise developmental decisions involving cell growth, differentiation and morphogenesis. This obviously would require a highly interactive molecular communication between cells and tissues for ensuring an error-free, reproducible, species-specific developmental path.

In recent years, the basic genetic network propelling the initial determinative steps is beginning to emerge. A number of genes and their morphogenic ligands involved in signalling pathways have been identified. In these studies, *Drosophila melanogaster*, owing to its relatively shorter life cycle, wealth of genetic and genomic information, and commonality of key developmental steps in a major class of animals, has emerged as one of the most important model systems for understanding the genetic control of development and other life processes, including human diseases and cancer. It has been estimated that about 75% of known human disease genes have DNA sequence homologues in the Drosophila genome[1]. Consequently, the fly is now being used routinely for unravelling the molecular basis of genetic disorders, including neurodegenerative disorders like Parkinson's, Huntington's, Alzheimer's disease and diseases influencing, ageing, immunity, diabetes and cancer.

Discovery of wingless and in turn *Wnt*

The wingless mutation was initially discovered in 1973 (ref. 2) from the screen of ethyl methanesulphonate mutagenized *Drosophila* population. It was the first member of a family of seven wingless genes found in *Drosophila*. Wingless is governed by a single recessive gene (wg^1) located on the second chromosome (2–21.9 cM). The distinct phenotypic characters of the wingless include absence of wings and halters with all combinations of two wings, one wing and no wings with and without one or both halters. Other significant phenotypes were duplication of the notum, partial or complete absence of mesothoracic bristles, absence or deformed scutellum, irregular arrangement of hairs, hemithoracic and thoraxless flies[3]. To explain the various phenotypes, it was hypothesized that the wg^1 mutation affected wing and halter development at multiple steps during wing specification in a cellular compartment-specific manner. Detailed studies[4] on the wing imaginal discs in wg^1 revealed a considerable increase in the number of dead cells, suggesting thereby that either wg^1 itself was inducing cell death or altering the process of programmed cell death. The cells escaping wg^1 action would yield a mirror image or would develop normally, whereas those undergoing degeneration or death would give distinct morphological variants.

Incidentally, wg^1 gene of *Drosophila* was found to be homologous to int^{-1} gene in mouse[5]. int^{-1} is a vertebrate oncogene that results in mammary tumour in mouse followed by insertion of Mouse Mammary Tumor Virus (MMTV)[6]. This observation led to a new nomenclature, *Wnt*, as the concatenation of *wg* and *int* (*wg* + *int* = *Wnt*). Following the connection between *wg*, *int* and cancer, the *Wnt* gene family received overwhelming attention and now more than hundred *Wnt* genes have been discovered in different organisms, starting from nematode to humans (The *Wnt* home page: http://www.stanford.edu-musse/).

Wingless determines body segmentation and segment polarity

Nusslein-Volhard and Wieschaus[7] studied the *Drosophila* larval cuticle pattern in wingless and discovered that it is a segment polarity gene. Its pattern of expression is responsible for the banding of cuticular dentricles and for determining segment polarity. As a result of their work, they were awarded the Nobel Prize in Physiology or Medicine in 1995 (http://en.wikipedia.ofg/wiki/wnt). It is now known that *Wnt* gene family secrete lipid-modified (palmitoylation) signalling protein about 350–400 amino acids long, characterized by a signal sequence and a cystein-rich sequence of 23 invariant cys residues[8]. The Wnt proteins are a major class of secreted morphogenic ligands which act on engrailed-expressing cells after the cellular blastoderm is formed and activate cell surface receptors, Frizzled, eventually releasing β-catenin from the cell membrane-bound protein complex. β-catenin travels to the nucleus and binds to DNA to turn on the expression of targeted genes involved in body segmentation. Recently, Swarup and Verheyen[9] reviewed the work on *Wnt/wg* signalling and concluded that the wingless gene product is a morphogenic ligand of profound importance which diffuses away from its source and interacts with targeted genes in a concentration-dependent manner.

The involvement of wingless gene product in establishing the pattern of development in the bodies of all multicellular organisms has been documented[10]. The *Wnt* gene family has also been implicated in the maintenance of adult tissue homeostasis. Further, the diverse phenotypic changes observed in different organisms following mutation/alterations in *wg/Wnt* signalling suggest that *wg* signals are pleotropic with effects that include mitogenic stimulation, cell fate specification and differentiation.

Wnt is involved in human diseases and cancer

The human genome is known to contain 19 *Wnt* genes and alterations in the signalling of these genes or loss of any of these genes has been associated with a number of medical conditions ranging

from CNS abnormalities and leukaemia to developmental changes such as embryonic lethality, female infertility, kidney and limb defects[1,8]. The autosomal recessive disorder *tetra-amelia* (loss of all the four limbs), a rare human genetic disorder is due to nonsense mutation in *Wnt*$^{-3}$ gene[11].

Incidentally, *Wnt*$^{-1}$ was also found to be an oncogene activated by MMTV in murine breast cancer. Subsequent studies in mammalian systems demonstrated a strong correlation between the levels of β-catenin and tumorogenesis. APC gene in humans is a tumour-suppressing gene in colon cancer[12]. It binds to β-catenin and therefore a mutation in either of these genes results in higher levels of β-catenin and initiation of tumour formation. It is now well established that activating mutations of *Wnt* signalling pathway are responsible for approximately 90% of colorectal cancer (http://www.stanford.edu/musse/pathway/wnt-cancer.html). Recent studies have also shown that any perturbation in *Wnt* signalling leading to changes in the level of β-catenin can result in excessive stem cell proliferation, predisposing the cells to formation of tumours[10,13]. Based on these findings, several therapeutic approaches involving the use of inhibitors of *Wnt*–β-catenin signalling through siRNA and antibodies against *Wnt* are being tested for cancer therapy[12,14].

To conclude, discovery of wingless mutant in *Drosophila* proved to be a landmark finding which led to: (i) Identification of a number of other *wg*/*Wnt* gene families in organisms across the animal kingdom, from nematodes to humans. (ii) Demonstration that *Wnt* gene product is involved in crucial signalling pathways affecting diverse developmental processes such as pattern formation, differentiation, tissue induction, axis specification and tumorogenesis. (iii) Establishment of association between alteration in *Wnt* signalling with a number of human diseases, including cancer. (iv) Innovative drug-mediated approaches for inhibition of aberrant *Wnt* signalling in cancer therapy.

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