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Application of Temperature-Sensitive Mutations to Oncogene Studies in *Drosophila*

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Recessive oncogenes are genetic functions important in the regulation of tissue growth and differentiation. These genetic functions are defined on the basis of the phenotype expressed by homozygotes. Defining the role of these genes in normal developmental and physiological processes is important to the development of accurate models of the normal regulation of growth and differentiation. *Drosophila* can be a good system to investigate the neoplastic mechanism of oncogenes and provide a greater understanding in the developmental progression of both invertebrates and vertebrates. The *lethal (2) giant larvae* gene is a recessive oncogene of *Drosophila* and temperature sensitive mutations of this gene have been isolated. Here, the application of temperature-sensitive mutations in *Drosophila* oncogene studies is discussed.

Key words : Lethal (2) giant larvae, Cadherin, Cell adhesion molecule, Intragenic complementation

INTRODUCTION

Drosophila melanogaster has been providing a good model for genetic and developmental studies because of its short life span, manipulatable genetics, and well-characterized embryonic and post-embryonic development (Mitchison and Sedat, 1983). During post-development there are two populations of cells; 1) non-dividing cells that are for the most part terminally differentiated, and 2) dividing cells. In *Drosophila* there have been identified at least 27 recessive genes which are capable of causing tissue-specific tumors when mutated (Gateff, 1994). Since only dividing cells express and proliferate the neoplastic state, the mutations producing subculturable neoplasms affect dividing cells such as the presumptive adult optic centers in the larval brain, the primordia for the adult integument (imaginal discs), the primordial gonial cells in the female and male gonads, and the hematopoietic cells (Gateff, 1978, 1982).

Temperature-Sensitive Mutations

A powerful tool for analyzing the effect of lethal mutations on developmental processes is temperature-sensitive (ts) lethal mutation (Suzuki, 1970). Temperature-sensitive mutations have been used in a variety of developmental studies in various organisms including *Drosophila* (Suzuki, 1970; Arking, 1975; Hanratty, 1984; Baek and Hanratty, 1996) and microorganisms (Prendergast *et al.*, 1996). In microorganisms, temperature sensitivity results from missense mutations that produce thermolabile polypeptide products (Jockusch, 1966). The genetic properties of temperature-sensitive mutations in *Drosophila* conform to the expectations of point mutants (Suzuki, 1970). The temperature-shift studies have been used to define temperature-sensitive periods of the gene action by determining the effect of shift from the permissive condition to the restrictive condition (upshift) or from the restrictive condition to the permissive condition (downshift) on the expression of the mutant phenotype (Fattig and Rickoll, 1972; Hanratty, 1984). Temperature-sensitive alleles at the known neoplastic loci are useful in analyzing the developmental, genetic, and biochemical aspects of neoplastic development in *Drosophila*. Since there are a number of known non-conditional mutants of *Drosophila* that develop neoplasms, these mutants define specific developmental genes and the non-conditional mutants can, in turn, be used to identify temperature-sensitive alleles of the same gene. Therefore, by using temperature-sensitive alleles of known neoplastic genes, the role of these genes in normal development can be defined.

The Characteristics of *Lethal (2) Giant Larvae*

The *l(2)gl, lethal (2) giant larvae*, has been described as the first recessive oncogene in *Drosophila* (Gateff,

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1978; Mechler, 1985) and its homologs have been found in mouse and human (Kuwabara *et al.*, 1994; Strand *et al.*, 1995). Both recessive non-conditional and conditional mutants for this gene have been isolated in Drosophila (Gateff, 1978; Hanratty, 1984; Baek and Hanratty, 1996) and the chromosomal segment containing the *l(2)gl* gene has been cloned (Mechler *et al.*, 1985). Sequence analysis of the cDNA clone suggests that it encodes either an 1114 or 1161 amino acid depending on the two possible initiation codons in the open reading frame (Lützelschwab *et al.*, 1987). The p127 tumor suppressor protein encoded by the *l(2)gl* gene is homologous to vertebrate cadherin cell adhesion molecules such as mouse P-cadherin and chicken L-CAM. Even though *l(2)gl* protein lacks the transmembrane domain present in cadherins, it contains an internally located signal sequence near the N-terminus. Immunocytochemistry studies have shown that *l(2)gl* proteins are localized at the cell surface, specifically at the junctions between proliferating cells (Klämbt et al., 1989). These findings suggest that *l(2)gl* protein is involved in cell-to-cell interaction.

The *l(2)gl* protein is found in a cytoskeletal complex containing a nonmuscle myosin type II heavy chain (zip), a serine kinase, a nucleosome-assembly protein-1 (NAP-1), and D-abelson (Kalmes *et al.*, 1996; Neumann *et al.*, 1996; Saha and Sinha, 1996). It has been suggested that the organization of this complex and its association with other unknown proteins are required for the cell proliferation (Jacobs *et al.*, 1996). Therefore, finding these unknown proteins and delineating the role of *l(2)gl* in this complex will clarify to understand the mechanisms of this cytoskeleton complex during cell proliferation.

Recessive mutant alleles of *l(2)gl* gene result in the overgrowth of presumptive adult optic centers and the imaginal discs of the affected larvae. The critical evidence that demonstrates the neoplastic nature of the tissue obtained from these larva was the ability to establish subculturable neoplasms when the affected tissues were cultured in adult hosts (Hanratty, 1984; Gateff and Schneiderman, 1974). The expression analysis for this gene and other Drosophila recessive lethal mutations has demonstrated that mutations in a number of different genes result in the overgrowth and neoplastic development of affected tissues (Gateff, 1994). The molecular and developmental analyses of these mutations are an active area of investigation. An important approach of the developmental analysis of gene expression in *Drosophila* is the isolation and analysis of temperature-sensitive alleles. Three temperature sensitive *l(2)gl* alleles have been isolated (Hanratty 1984; Baek and Hanratty, 1996) using ethylmethane sulfonate (EMS), which is a chemical mutagen (Fig. 1; Table I). And the temperature dependent expression of the neoplastic development of the wing imaginal discs in these alleles has been demonstrated (Hanratty, 1984; Baek and Hanratty, 1997). In addition,

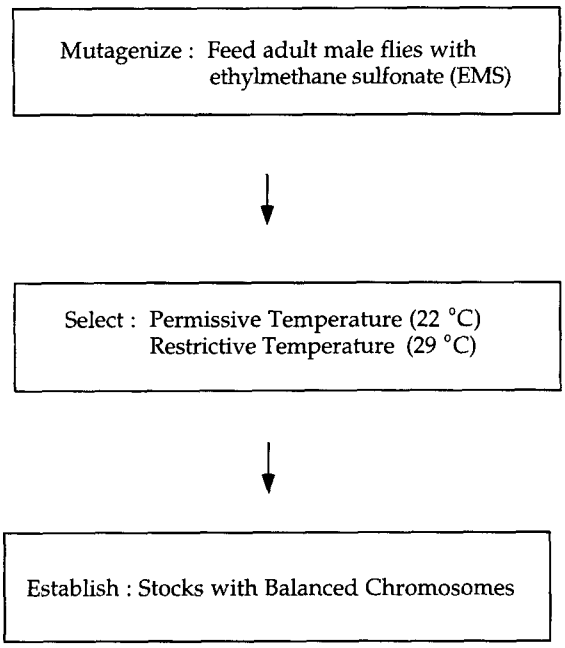

Fig. 1. The general scheme for the isolation of temperature-sensitive mutations in *Drosophila*. Newly induced *l(2)gl* alleles become lethal at 29°C and viable at 22°C. To establish temperature-sensitive *l(2)gl* stocks, a balancer chromosome was used to prevent the recovery of unwanted recombination during the isolation procedure.

Table 1 The generation of temperature-sensitive *l(2)gl* alleles by EMS mutagenesis

The Number of Chromosomes Tested	Newly Induced *l(2)gl* Alleles	Viable at 22°C
2,969	6	2*
2,500	3	1**

*named $l(2)gl^{ts1}$ and $l(2)gl^{ts2}$ (Haratty, 1984)
**named $l(2)gl^{ts3}$ (Baek and Hanratty, 1996)

it has been shown that the intragenic (trans-allelic) complementation may occur between temperature-sensitive *l(2)gl* alleles (Baek, 1996), supporting the finding that *l(2)gl* proteins homo-oligomerize (Jacobs, 1996).

Future Directions

A detailed analysis of recessive mutations at the temperature-sensitive *l(2)gl* locus on both the genetic and molecular levels will help to elucidate the unresolved questions regarding the functions of this gene during proliferative development. The effort also has been initiated for finding homologs in other organisms such as zebrafish, Xenopus, and chick. The metastasis of *Drosophila* cells is similar to the metastasis of some mammalian (including human) tumors not only at the biochemical level but the cellular level, suggesting that similar mechanisms may play a role in this process in diverse organisms. Therefore, the investigation of the mechanism

for neoplastic development in invertebrates will help to create drugs that target to this mechanism in human tumors.

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