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raf Proto-oncogene is Involved in Ultraviolet Response in *Drosophila*

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Raf-1, a cytoplasmic serine/threonine protein kinase, serves as a central intermediate in many signaling pathways in cell proliferation, differentiation, and development. In this study, we investigated that *D-raf*, *Drosophila* homolog of the human *c-raf-1*, is involved in ultraviolet (UV) responsive events by using hypomorphic mutant *D-raf*^{C110} and *Draf-lacZ* transgenic fly. At first, effect of UV damage on the survival of wild-type and *D-raf*^{C110} strains was examined. In terms of $1/LD_{50}$ value, the relative ratio of UV sensitivities of wild-type versus *D-raf*^{C110} strain was 1 : 2.2. By using quantitative β-galactosidase activity analysis, transcriptional activity of the *D-raf* gene promoter was also examined in UV-irradiated *Draf-lacZ* transgenic larvae. UV irradiation increased the expression of *lacZ* reporter gene in *Draf-lacZ* transgenic fly. However, in *D-raf*^{C110} strain the transcriptional activity of *D-raf* gene promoter by UV irradiation was extensively reduced. Results obtained in this study suggest that D-raf plays a role in UV response, leading to better survival of *Drosophila* to UV damage.

Treatment of cell with environmental stress including DNA damaging agents, UV irradiation, ionizing radiation, alkylating agents or bulky adduct formers, causes massive regulatory changes that, by and large, mimic the proliferating response induced by phorbol ester or growth factors (Herrich et al., 1992; Kasid et al., 1996). UV light enhances the transcriptional activity of several genes, e.g., human immunodeficiency virus type 1 (HIV-1), collagenase, c-*fos*, and metallothionein (Stein et al., 1989). UV-induced transcriptional activation of c-*fos*, HIV-1 and collagenase genes is known to be mediated through same enhancer elements responding to phorbol ester and growth factor (Stein et al., 1989). UV irradiation not only augments the activity of pre-existing transcription factors, such as Fos, Jun, AP1, and NF-kB (Devary et al., 1993; Sachsenmaier et al., 1994a), but activates new synthesis of genes for repairing of DNA damage (Stein et al., 1989). Two distinct signal transduction pathways for the UV response have been suggested (Mount, 1996). The first proposed pathway is that DNA damage generates the primary signal which leads to the induction of UV responsive genes (Karin and Herrlich, 1989; Holbrook and Fornace, 1991; Herrich et al., 1992). The second is that the pathway initiated in an extranuclear compartment (Devary et al., 1992; Radler-Pohl et al., 1993; Sachsenmaier et al., 1994b). In both pathways, the signaling component activates the activity of transcription factors, leading to the transcriptional increment of specific target genes (Mount, 1996).

Raf-1, cytoplasmic serine/threonine protein kinase, mediates the transmission of mitogenic signals initiated at the cell membrane to the nucleus, resulting in the activation of transcription factors that regulate cell growth and proliferation (Kolch et al., 1991). *D-raf*, *Drosophila* homolog of human *c-raf-1*, has been cloned and also shown to be required in the regulation of cell proliferation and differentiation (Nishida et al., 1988; Ambrosio et al., 1989; Hata et al., 1994). On the other hand, Raf-1 kinase has also been proposed to be an obligatory bottle neck shared by UV, phorbol ester and other growth factors (Rapp, 1991; Kyriakis et al., 1992; Sachsenmaier et al., 1994b). In deed, Radler-Pohl et al. (1993) demonstrated that UV-induced signal transduction depends on the activation of Raf-1 kinase in HeLa tk$^-$ cells.

Most evidences for the existence of UV signaling pathway have been obtained by using the mammalian cells *in vitro*. However, it is not tested yet whether Raf really involves in UV signaling pathway *in vivo* or UV signaling pathway is conserved in between *Drosophila* and mammal. In this study, by using transgenic fly carrying *Draf-lacZ* fusion gene and hypomorphic mutant *D-raf*^{C110} strain, we demonstrate that *D-raf* gene is involved in UV response in *Drosophila*.

Materials and Methods

Fly stocks

Fly culture and crosses were performed according to

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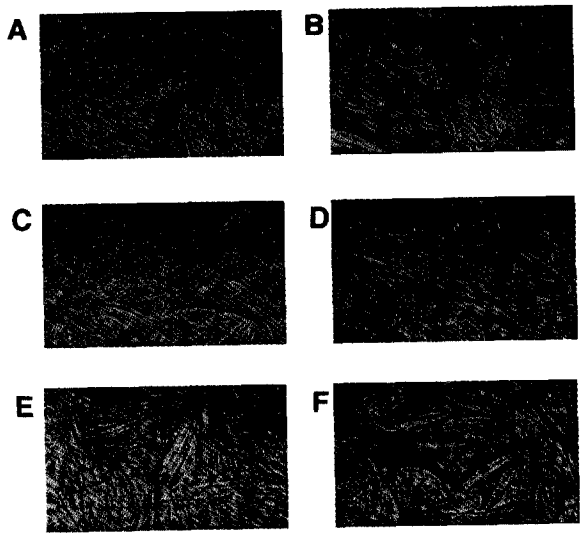

Fig. 1. Morphological effects of LPA in E63 cells. The E63 cells cultured in DMEM containing 10% horse serum were seeded onto gelatin coated 6-well plates at the density of 3×10^4 cell/well. After 48 h in culture, the medium was switched to ITS medium (B), or ITS supplemented with LPA 2 μg/ml (4.6 μM; C), 5 μg/ml (11.5 μM; D), 10 μg/ml (23.0 μM; E), 15 μg/ml (34.5 μM; F), or 10% horse serum (A). After 4 d in culture with daily changing the medium, the cells were fixed and stained with Hematoxylin-Eosin.

then daily changed the medium with serum-free ITS medium with or without LPA. When myoblasts were cultured in the complex medium, they started to fuse within 3 d after plating, and differentiated to form myotubes (Fig. 1A and Fig. 2). When myoblasts were grown in the serum-free ITS medium without LPA, the proliferation was largely restricted and the cell number was not much increased because insulin is a unique proliferation-promoting molecule in ITS medium (Fig. 1B and Fig. 2). The addition of LPA in ITS medium markedly increased the cell number by 2 to 4 fold and its effective concentration was higher that 5 μg/ml (11.5 μM) (Fig. 1 and Fig. 2A). This is about same dose (6.5 μg/ml) required to induce cellular reponses such as DNA synthesis and proliferation in fibroblasts (Van Corven et al., 1989, 1993). Addition of LPA to the complex medium containing horse serum had no effect in cell proliferation and percent fusion (data not shown). This may be due to the fact that serum contains both LPA in the range of 0.87-8.6 μg/ml and various growth factors (Eichhoits et al., 1993). However, concentrations higher than 20 μg/ml of LPA caused cell lysis because LPA is a polar lipid and has a detergent-like activity (data not shown).

LPA effect on myoblast differentiation.

In the terminal differentiation process, myoblasts were fused and formed myotubes, and expressed muscle-specific proteins such as myosin heavy chain (MHC), tropomyosin, troponin and muscle creatin kinase. To elucidate the effect of LPA on E63 myoblast differentiation, myoblasts were cultured in ITS medium con-

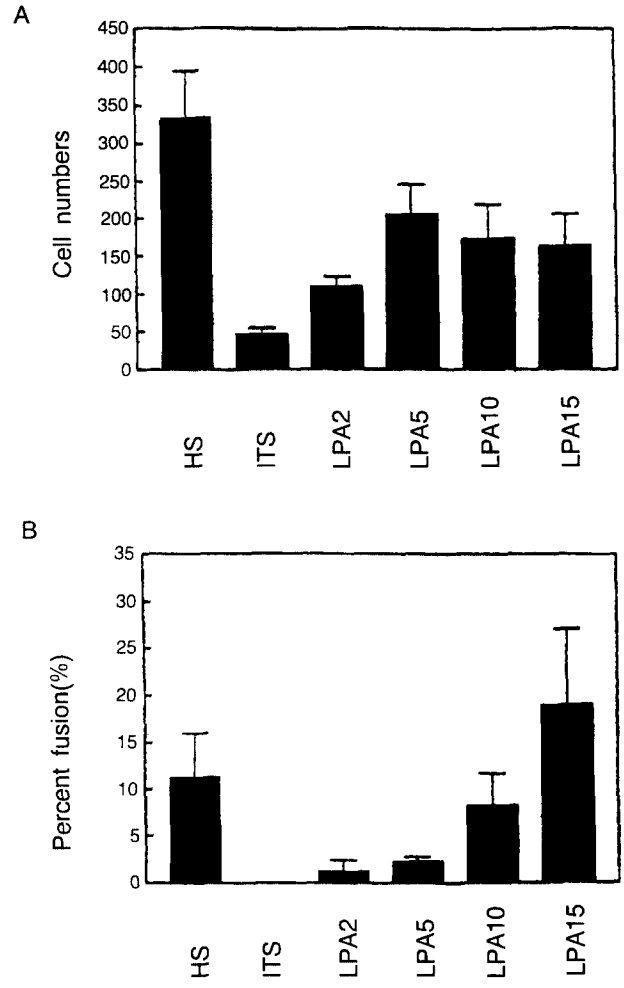

Fig. 2. Effect of LPA on proliferation and differentiation of E63 cells. (A) Dose dependent stimulation of myoblast proliferation by LPA. (B) Effects of fusion stimulation in E63 cells by LPA. E63 cells were cultured in DMEM containing 10% horse serum for 2 d and then cultured for 4 d in ITS medium (ITS), with LPA 2 μg/ml (ITS2), 5 μg/ml (ITS5), 10 μg/ml (ITS10), 15 μg/ml (ITS15), or 10% horse serum (HS). At 4 d after daily changing the media, the cells were fixed and then stained with Hematoxylin-Eosin. The percent fusion was determined by the ratio of the number of nuclei in myotubes with three or more nuclei divided by the total number of nuclei as seen at X 200. Ten independent fields were chosen for each dish.

taining LPA. Myogenic differentiation was indicated by MHC expression and percent fusion. The expression of MHC was examined by Western blot analysis using anti-MHC mouse monoclonal antibody (MF20). In culture containing 10% horse serum, E63 cells normally expressed MHC at 4 d after plating and its expression was gradually increased during differentiation (Fig. 5). In serum-free ITS medium, the expression of MHC and cell fusion were strongly suppressed (Fig. 2 and Fig. 5). LPA in ITS medium appeared to stimulate myogenic differentiation in a dose-dependent manner up to 15 μg/ml in which the expression of MHC and myoblast fusion were markedly increased (Fig. 2B and Fig. 5).

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