

Genetic Screen for Genes Involved in Chk2 Signaling in *Drosophila*

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Chk2 is a well characterized protein kinase with key roles in the DNA damage response. Chk2 is activated by phosphorylation following DNA damage, and relays that signal to various substrate proteins to induce cell cycle arrest, DNA repair, and apoptosis. In order to identify novel components of the Chk2 signaling pathway in *Drosophila*, we screened 2,240 EP misexpression lines for dominant modifiers of an adult rough eye phenotype caused by Chk2 overexpression in postmitotic cells of the eye imaginal disc. The rough eye phenotype was suppressed by mutation of the ATM kinase, a well-described activator of Chk2. Twenty-five EP modifiers were identified (three enhancers and 22 suppressors), none of which correspond to previously known components of Chk2 signaling. Three EPs caused defects in G2 arrest after irradiation with incomplete penetrance when homozygous, and are likely directly involved in the response to DNA damage. Possible roles for these modifiers in the DNA damage response and Chk2 signaling are discussed.

INTRODUCTION

When the nuclear DNA is damaged, metazoan cells activate a complex DNA damage response which delays cell cycle progression and promotes the repair of damaged DNA, and can also lead to apoptosis (Sancar et al., 2004). Failure of this response results in the incorporation of mutations, potentially leading to uncontrolled proliferation and initiation of tumor formation. At least two classes of protein kinases are known to mediate cellular responses to DNA damage. The first is the ATM and ATR group which consists of proteins with homology to the phosphatidylinositol-3-kinase (PI3-K) family of kinases. The second group of kinases includes Chk1 and Chk2 which can be activated by ATM and ATR, and transduce the signal via phosphorylation of a variety of substrate proteins.

Studies in mammalian cell culture systems, and in yeast, have identified activators and targets of Chk2 (Antoni et al., 2007). Human Chk2 is activated by DNA damaging agents such as ionizing radiation (IR) and UV, and also by DNA replication block by hydroxyurea (HU). Activation of Chk2 by IR is dependent on ATM, whereas activation of Chk2 by UV and HU

treatment is ATM-independent, and likely mediated by ATR. Upon activation, Chk2 can phosphorylate a number of DNA repair, tumor suppressor and cell cycle proteins including BRCA1, p53, PML, E2F, CDC25A, and CDC25C. BRCA1 is a tumor suppressor gene mutated in 50% of familial breast cancer patients and plays an important role in DNA repair. Chk2 can also phosphorylate and stabilize p53, a tumor suppressor that induces apoptosis or G1 arrest in response to DNA damage. Phosphorylation of PML and E2F can induce apoptosis by a p53-independent pathway (Stevens et al., 2003; Yang et al., 2002). There are three different CDC25 protein phosphatases, CDC25A, CDC25B, and CDC25C, all of which can be phosphorylated by Chk2 (Antoni et al., 2007). CDC25A is involved in blocking the progression through S phase, whereas CDC25B, and CDC25C can induce G2 arrest. Phosphorylated forms of CDC25A are rapidly degraded by the ubiquitin proteasome pathway, whereas phosphorylation of CDC25C leads to its sequestration and inactivation. Thus, via phosphorylation of its target proteins, activated Chk2 promotes critical processes of the DNA damage response including apoptosis, inhibition of the G2/M and G1/S transitions, and progression through S-phase.

Since the identification of the *Drosophila* p53 homolog (Brodsky et al., 2000; Ollmann et al., 2000), *Drosophila* has emerged as a useful model organism for studying the DNA damage response. Studies in the larval imaginal disc showed that, as in human cells, irradiation inhibits both the progression of S-phase and the G2/M transition, and induces apoptosis (Song, 2005). A G1 arrest was also induced, but it was not as robust in the imaginal disc as in human cells. Studies have examined the effects of null alleles of the ATM (*tefu*), ATR (*mei-41*), Chk2 (*mnk*), Chk1 (*grapes*), p53, and CDC25 (*string*) homologs of *Drosophila*. ATR and Chk1 were not required for IR-induced apoptosis, while ATM, Chk2, and p53 were indispensable. ATR, Chk1, Chk2, and CDC25, but not ATM and p53, were required for maintaining the IR-induced G2 arrest. These results suggest that the DNA damage response is highly conserved between fly and human.

Many genes involved in Chk2 signaling are involved in tumorigenesis and we were interested in identifying novel tumor suppressors or oncogenes involved in Chk2 signaling. To identify genes involved in the Chk2 signaling pathway, we generated transgenic flies that overexpress Chk2 in the eye using the

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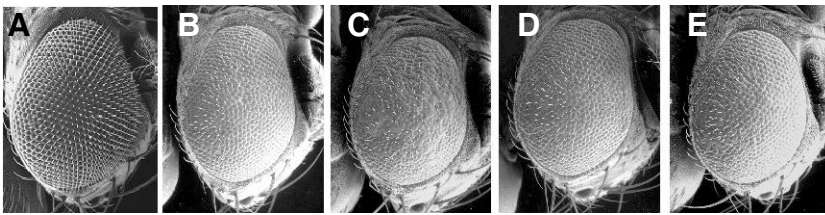


Fig. 1. Overexpression of Chk2 induced a rough eye phenotype. (A–E) Scanning electron micrographs of eyes from wild type, Oregon R (A), GMR-Gal4/+ (B), GMR-Gal4/+; UAS-Chk2/+ (C), GMR-Gal4/+; UAS-Chk2/GMR-p35 (D), GMR-Gal4/+; UAS-Chk2/ATM⁻ (E).

GAL4-UAS system. In GMR-GAL4;UAS-Chk2 transgenic lines, the expression of Chk2 in the eye is controlled by the GAL4 transcriptional activator, driven by the eye-specific GMR promoter. A modifier screen was performed using 2,240 EP lines (Rørth, 1996). We identified three enhancers and 22 suppressors of Chk2 dependent rough eye, and examined whether they are directly involved in DNA damage response.

MATERIALS AND METHODS

Fly stocks and construction of transgenic flies

Flies were grown on a standard cornmeal medium at 25°C. Wild type and kinase dead (D286A) mutant forms of *Drosophila* Chk2 (Large form) cDNAs were obtained by reverse transcription (RT)-PCR amplification from the wild type, Oregon R flies. cDNAs were cloned into pUAST vector and transgenic lines were generated by conventional gene transfer methods. EP lines were obtained from Exelixis. UAS-Notch was a gift of Dr. S. Artavanis-Tsakonas (Harvard Medical School). GMR-p35 was obtained from Bloomington *Drosophila* Stock Center and the ATM mutant fly was described previously (Song et al., 2004).

Scanning electron micrographs of adult eye

Scanning electron micrographs of eyes were performed as described previously (Song et al., 2004).

EP screen

GMR-GAL4; UAS-Chk2 virgin female flies were mated to males from the EP collection. F1 progeny were scored for modification of the GMR-GAL4;UAS-Chk2 rough eye phenotype. All crosses were carried out at 25°C.

Cell cycle arrest upon irradiation

To analyze radiation-induced cell cycle arrest, wandering third instar larvae were mock treated or irradiated in a Cs¹³⁷ gamma irradiator (MDS Nordion, Canada) at 40 Gy. After 1 h, wing imaginal discs were dissected and fixed in 4% paraformaldehyde in PBS for 30 min at room temperature. Discs were washed in PBS and 0.3% Triton X-100 (PBT), and blocked in PBT and 5% goat serum (PBTN). Discs were incubated in a 1:250 dilution of a rabbit anti-phospho-histone H3 antibody (Upstate Biotechnology) in PBTN overnight at 4°C. They were incubated with a 1:250 dilution of a rhodamine conjugated goat anti-rabbit antibody (Jackson ImmunoResearch Laboratories, INC) in PBTN and observed with an Olympus IX71 fluorescence microscope.

RNA purification and RT-PCR

To determine the transcript levels of *misexpression suppressor of Ras4* (MESR4), *hephaestus*, and HLHm7 in EP(2)0386, EP(3)3574, and EP(3)3587, respectively, we isolated total RNA from adult female or third instar larvae using easy-spin™ Total RNA Extraction Kit (iNtRON Biotechnology, Korea). RNA was reverse transcribed using AccuScript reverse transcriptase

(Stratagene, USA) and 0.5 mM oligo(dT)₁₂₋₁₈ as a primer. cDNA products were amplified with Taq DNA polymerase in the presence of the specific primers for each gene. Primers were designed to amplify a region that covers intron, except for HLHm7 that does not contain intron.

RESULTS AND DISCUSSION

Overexpression of Chk2 induces rough eye phenotype

The development of the *Drosophila* compound eye requires intricate regulation of various cellular processes including cell cycle, cell growth, cell proliferation, differentiation, and programmed cell death. Perturbation of these processes, for example by overexpression of a relevant gene, can result in a rough or small eye phenotype. Ectopic expression of *Drosophila* Chk2 in fly eyes using GMR-GAL4 induced a rough eye phenotype (Fig. 1C). The rough eye phenotype was dependent on the kinase activity of Chk2 since it was not induced by overexpression of a kinase-inactive mutant of Chk2 (data not shown).

The Chk2-induced rough eye phenotype was suppressed by the expression of the general apoptosis inhibitor p35 from baculovirus (Fig. 1D; Beidler et al., 1995), suggesting that the rough eye phenotype was caused by apoptosis. This is consistent with the observation that Chk2 plays a role in IR-induced apoptosis in the larval wing discs (Xu et al., 2001). Moreover, the rough eye phenotype was suppressed by mutation of ATM (Fig. 1E), a well known activator of Chk2, indicating that the rough eye phenotype has physiological relevance.

EP screen

To identify novel components of the Chk2 signaling pathway, we performed a modifier screen using EP lines generated by Rørth (1996). Each EP contains a transposable element consisting of a GAL4 binding site and a basal promoter to direct the expression of the gene located downstream of the EP insertion site. Since transposable elements insert preferentially into 5' ends of genes, EP insertion causes over- or mis-expression of affected genes. EP lines were crossed with GMR-GAL4;UAS-Chk2 flies and progeny were scored for eye phenotype. Of 2,240 GMR-GAL4;UAS-Chk2/EP lines screened, 104 displayed suppression of the Chk2-induced rough eye phenotype, while 121 showed enhancement. Some of the EPs (4% of the collection) can generate eye phenotypes when overexpressed in the eye (Rørth et al., 1998), suggesting that perhaps the enhancement of Chk2 eye phenotype by 121 EPs might be independent of Chk2. Therefore, we determined whether any of the EPs enhancing the rough eye phenotype cause an eye phenotype in GMR-GAL4 flies. We found that 114 EP lines generated a rough eye phenotype when crossed to GMR-GAL4, and these lines were not pursued any further since the enhancement of Chk2-induced roughness by those EPs is probably caused by perturbation of some aspect of eye development other than Chk2-related processes.

To determine whether the modifiers specifically affected the

Table 1. EP lines that modified the rough eye phenotype of GMR-GAL4; UAS-Chk2

EP line	Genes nearby	Functional information
Enhancers		
EP(2)2124	ND*	
EP(3)0417	ND	
EP(3)0863	<i>ballchen</i>	Protein kinase that phosphorylates nucleosomal histone H2A (Aihara et al., 2004)
Suppressors		
EP(X)1353	<i>NFAT</i>	Identified in genetic screens (Huang and Rubin, 2000; Pena-Rangel et al., 2002)
EP(X)1607	CG6961	Contains RNA binding domain (Schultz et al., 1998)
EP(X)1618	ND	
EP(2)0386	<i>misexpression suppressor of Ras4</i>	Contains PHD domain (Bienz, 2006)
EP(2)1027	<i>calmodulin</i>	Binds calcium, involved in mitotic spindle organization (Goshima et al., 2007)
EP(2)2056	CG33960	Unknown
EP(2)2081	CG30492	Identified as a sensory organ precursor gene in microarray analysis (Reeves and Posakony, 2005)
EP(2)2161	ND	
EP(2)2263, EP(2)2576	<i>smell impaired 21 F</i>	Identified as a mutation affecting olfactory avoidance response (Anholt et al., 2003)
EP(2)2522	CG15161	Identified in a genetic screen (Schulz et al., 2004)
EP(3)0703	CG8165	Contains jumoni C-domain (Klose et al., 2006)
EP(3)0772	<i>SNF4Aγ</i>	Isoform of γ -subunit of AMP-activated protein kinase, mutant shows progressive neurodegeneration and neuronal cell death (Tschäpe et al., 2002)
EP(3)0902	<i>kayak</i>	<i>Drosophila</i> homolog of Fos, regulates cyclin B expression and the G2-M transition of the cell cycle (Hyun et al., 2006)
EP(3)1105, EP(3)3541	<i>trithorax</i>	Together with polycomb group proteins, maintains stable transcript patterns of many developmental regulators (Schuettengruber et al., 2007)
EP(3)1202	<i>murashka</i>	Identified as a mutant that shows defects in learning and memory (Dubnau et al., 2003)
EP(3)3289	<i>slamdance</i>	Identified as a "bang-sensitive" mutant that shows increased seizure susceptibility (Zhang et al., 2002)
EP(3)3377	<i>melted</i>	Contains PH (Pleckstrin Homology) domain, regulates cell growth and fat metabolism (Teleman et al., 2005)
EP(3)3574	<i>hephaestus</i> ,	Polypyrimidine tract binding protein, involved in wing development (Dansereau et al., 2002)
	CG2003	Identified as a transcript that is enriched in male stem cells by microarray analysis (Terry et al., 2006)
EP(3)3587	<i>HLHm7</i>	Contains DNA binding domain and is a part of the Enhancer of split complex required during neurogenesis for neural fate determination (Bray, 1997)
EP(3)3685	<i>short spindle 2</i>	Identified as a gene whose knockdown by RNAi results in a short spindle (Goshima et al., 2007)

*ND, The flanking sequence was not identified or the BLAST search did not result in specific match to the genomic DNA.

Chk2-induced rough eye phenotype, we determined whether they could modify the rough eye phenotype caused by misexpression of Notch, a gene that is not involved in Chk2 signaling. Notch is a regulator of differentiation, proliferation, and apoptosis, which is not known to be directly involved in the DNA damage response. We also tested whether Chk2- or Notch-induced rough eye phenotypes are suppressed by mutation of Notch or Chk2, respectively. We did not observe suppression in either case (data not shown), indicating that Chk2 and Notch do not have a genetic interaction that contributes to the rough eye phenotype. We crossed the 111 EPs that modified the Chk2-induced rough eye phenotype (104 suppressors and 7 enhancers) to UAS-Notch flies and scored the progeny for eye phenotypes. The screening of eye phenotypes caused by the EPs in GMR-GAL4 flies and UAS-Notch flies reduced the number of Chk2 modifiers to 22 suppressors and 3 enhancers (Table 1).

Chk2 enhancers

Three EP lines, EP(2)2124, EP(3)0417, and EP(3)0863, enhanced the rough eye phenotype induced by Chk2 but not by Notch. We obtained the flanking sequences of the EP elements

from FlyBase (Wilson et al., 2008), except for those of EP(2)2124 which were not available, and aligned them to the available *Drosophila melanogaster* genomic sequences from FlyBase using BLAST. The flanking sequence of EP(3)0417 could not be mapped to a specific genomic sequence. EP(3)0863 is located at the 5' of the *ballchen* gene (also known as NHK-1, nuclear histone kinase-1) in the same orientation as the transcription of *ballchen*. *ballchen* is a homolog of hVRK1 (vaccinia related kinase) and was identified as a histone kinase that can phosphorylate nucleosomal histone H2A (Aihara et al., 2004; Nichols and Traktman, 2004). Human VRK1 can phosphorylate and stabilize p53, but independently of Chk2 kinase (Vega et al., 2004). hVRK1 does not phosphorylate and activate Chk2, and seems not to be activated by DNA damage, indicating that VRK1 is not directly involved in the DNA damage response. Since Chk2 and VRK1 can both activate p53, the enhancement of the Chk2 phenotype by EP(3)0863 might result from activation of their common target, p53.

Chk2 suppressors

Of 104 EP suppressors tested, 22 suppressed the Chk2-

induced rough eye phenotype, but not the Notch-induced phenotype. The flanking sequences of EP(X)1618 and EP(2)2161 could not be matched to specific genomic sequences, but the other 20 EP lines were successfully mapped to 18 loci (Table 1). None of the 19 genes closely located to these EPs has been previously identified as a Chk2 regulator. The absence of known regulators of Chk2 is likely due to the fact that we used a misexpression screen. Most known Chk2 regulators are positive regulators that might have been identified as enhancers in this screen, but would have induced a rough eye phenotype when crossed to GMR-GAL4, and been excluded. For example, p53 overexpression induces rough eye phenotype and Chk2 overexpression enhances the p53-induced rough eye (Brodsky et al., 2004).

The genes located close to the 20 EP insertions belong to various functional categories including transcription regulation, RNA binding, and cell proliferation (Table 1). The connection to Chk2 signaling is not obvious for some genes, including those with no known function, while links can be inferred for others. For example, *calmodulin* and *short spindle 2* were isolated as regulators of spindle function (Goshima et al., 2007) and might act downstream of Chk2 to regulate the cell cycle progression. Proteins with DNA binding [*misexpression suppressor of Ras4* (MESR4) (Bienz, 2006)] and RNA binding domains [CG6961 (Schulz et al., 1998), and *hephaestus* (Dansereau et al., 2002)], and a predicted histone demethylase [jumonji C-domain protein CG8165 (Klose et al., 2006)], may contribute to changes in gene expression that occur downstream of Chk2 signaling in response to DNA damage. Interestingly, mutants of many genes [*smell impaired 21 F* (Anholt et al., 2003), *SNF4A γ* (Tschape et al., 2002), *murashka* (Dubnau et al., 2003), *slam-dance* (Zhang et al., 2002), and *HLHm7* (Bray, 1997)] display neurological abnormalities. Increasing attention has been paid to the role of DNA damage response in neurological disease (Kulkarni and Wilson, 2008). For example, mutations in the ATM gene in humans result in ataxia telangiectasia with progressive cerebellar ataxia, increased sensitivity to ionizing radiation, and a strong predisposition to cancer.

Screen for direct involvement in DNA damage response

The EP insertions that suppressed the Chk2-induced phenotype might directly affect the DNA damage response. To determine whether the overexpression of a gene near the insertion site of EP affects the DNA damage response, we used engrailed-GAL4, an expression construct that is active in the posterior region of the wing disc. Engrailed-GAL4 was crossed with EP flies of interest and progeny were scored for apoptosis and cell cycle arrest in response to DNA damage at the third instar larvae stage. The anterior region of the wing disc served as an internal negative control. The third instar larvae of engrailed-GAL4/EP were irradiated with 40 Gy and the wing discs were dissected and stained with acridine orange or phospho-histone H3 to detect apoptotic or mitotic cells, respectively. None of the larvae showed defects in IR-induced apoptosis or cell cycle arrest in the posterior part of the wing disc compared to the anterior region (data not shown). Interestingly, in some EP/ engrailed-GAL4, slightly increased number of mitotic cells was observed upon irradiation throughout the wing disc compared to the wild type (data not shown) leading to the following experiments.

Besides the overexpression of the genes near the point of the EP-element insertion, the suppression observed in the screen could result from inactivation of the genes by the EP. If the suppression was due to inactivation, larvae homozygous for the EP might show defects in DNA damage response. When the third instar larvae homozygous viable for EPs were tested

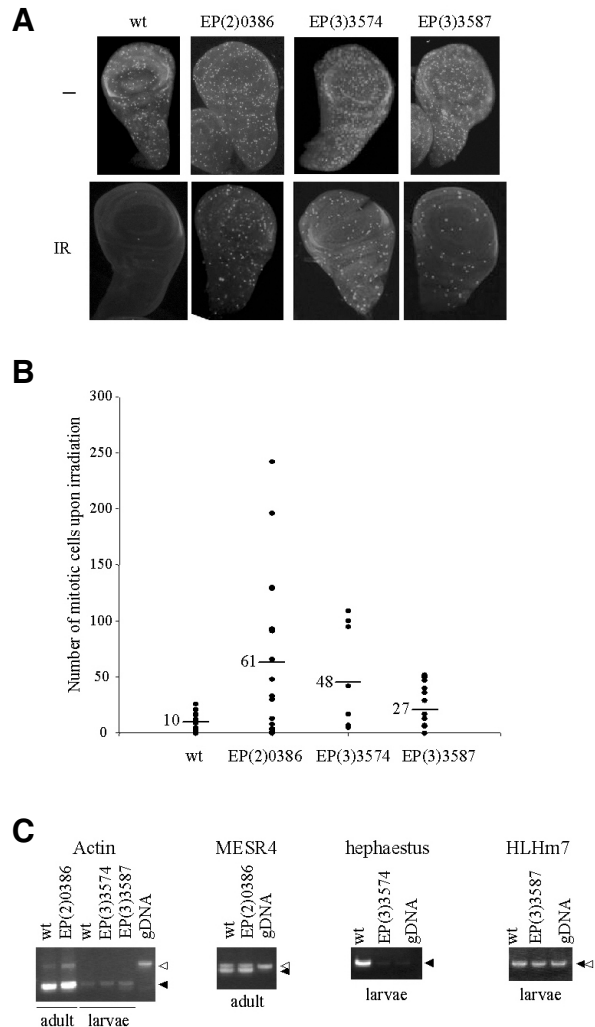


Fig. 2. EP(2)0386, EP(3)3574, and EP(3)3587 are involved in IR-induced G2 arrest. The third instar larvae of wild type or EP homozygotes were irradiated with 40 Gy and 1 h later, wing discs were dissected and stained with phospho-histone H3 antibody. (A) Representative wing discs are shown. (B) The number of mitotic cells in a wing disc from the 15 (wild type), 18 [EP(2)0386], 8 [EP(3)3574], and 11 [EP(3)3587] third instar larvae upon irradiation are shown. Mean values for each genotypes are indicated. (C) Total RNA was isolated from homozygous adult [wild type and EP(2)0386] and homozygous larvae [wild type, EP(3)3574, and EP(3)3587]. cDNA was generated and PCR amplification was performed. The PCR product from the cDNA and genomic DNA (gDNA) are indicated as \blacktriangle and \triangleleft , respectively. The PCR product of *hephaestus* genomic DNA is 7.6 kb longer than that of cDNA and is not shown. The relative location of primers are indicated in Fig. 3.

for IR-induced apoptosis, none showed defects (data not shown). However, with regard to IR-induced G2 arrest, significant numbers of mitotic cells were observed in three EP lines after IR treatment (Fig. 2A). The average number of mitotic cells in wild type wing discs after irradiation was 10 ($n = 15$), while the average number in EP(2)0386, EP(3)3574, and EP(3)3587 wing discs was 61 ($n = 18$), 48 ($n = 8$), and 27 ($n = 11$), respectively (Fig. 2B). Between 27 and 38% of the wing discs had a similar number of mitotic cells as wild type (be-

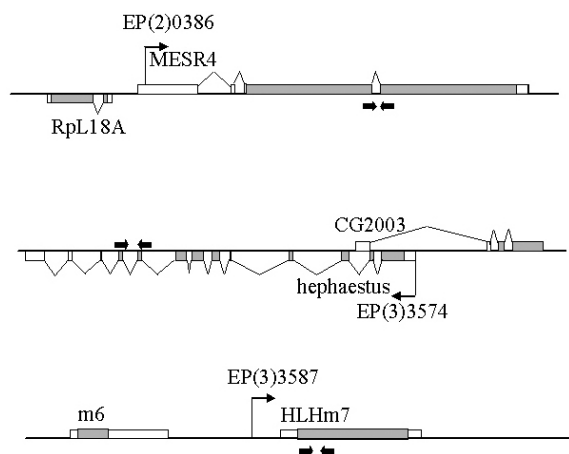


Fig. 3. Diagrams of the genomic regions indicating the insertion sites of the EPs in the three lines that showed defects in IR-induced G2 arrest. Genes transcribed from the top strand are shown as boxes above the line, while the genes transcribed from the bottom strand are shown below the line. The exon and intron structure is shown and indicated as a box and a line, respectively. The arrows indicate the EP element and the direction of the arrow points toward the direction of transcription driven by its promoter and enhancer sequences. The coding region is indicated as a shaded box. The approximate locations of the primers used for RT-PCR in Fig. 2. are indicated as black arrows. CG2003 and *hephaestus* contain more than one transcription start sites and only the transcripts whose start site is closest to the EP insertion sites are shown.

tween 0 and 10) and the number of mitotic cells varied depending on the animals. This observed incomplete penetrance suggests that the IR-induced G2 arrest in these mutants were affected by environmental and/or experimental conditions. The insertion sites and the orientation of these three EPs are indicated in Figure 3 and the genes they might affect are discussed below.

EP(2)0386

EP(2)0386 was previously isolated as a suppressor of a Ras1-dependent rough eye phenotype and designated as misexpression suppressor of RAS1^{V12}, *MESR4* (Huang and Rubin, 2000). The EP is inserted at the 5' end of the coding region of *MESR4*, which encodes a novel protein of 2171 amino acids. Analysis of *MESR4* using "SMART search" (Schultz et al., 1998) revealed nine zinc fingers and a PHD (plant homeodomain) zinc-finger-like motif. PHD proteins are nuclear and suggested to have nucleosome binding activity (Bienz 2006) implying that *MESR4* might be a nuclear protein capable of regulating gene expression.

EP(3)3574

EP(3)3574 disrupts two genes which are transcribed in opposite directions: it is inserted in the 5' end of *hephaestus* gene, and in the intron of a novel gene, CG2003 (Fig 3). Although proteins homologous to CG2003 have not been identified in the human genome, *hephaestus* is highly homologous to PTB (polypyrimidine tract-binding protein). PTB binds specifically to the polypyrimidine tract of the 3' splice site region of introns and regulates splicing (Garcia-Blanco et al., 1989). Interestingly, alternative splicing has been suggested as a mechanism to inactivate Chk2 in cancer (Staalesen et al., 2004), and knock-down of PTB suppresses ovarian tumor cell growth (He et al., 2007). It seems possible that PTB might regulate alternative

splicing of Chk2, thereby regulating its activity.

EP(3)3587

EP(3)3587 is located in the 5' end of HLHm7, one of seven clustered Enhancer of split [*E(spl)*] genes (m8, m7, m5, m3, mβ, mγ, and mδ). Hairy and *E(spl)* genes (Hes genes) are transcription regulators that can form homo- and hetero-dimers through basic helix-loop-helix domain. A number of human proteins are structurally similar to Hairy and *E(spl)* complex proteins (Davis and Turner, 2000; Fischer and Gessler, 2007), and sequence analysis by Ensembl and NCBI BLAST suggested that human Hes1, Hes4 and Hes6 are closely related to HLHm7. Constitutive expression of Hes1 reduces the proliferation of hematopoietic progenitor cells associated with upregulation of cell cycle inhibitor p21 (Yu et al., 2006). These results suggest the possibility that the human HLHm7 homolog might be involved in Chk2 signaling by regulating the cell cycle progression. Hes1 is known to be regulated by activation of Notch signaling (Davis and Turner, 2000), while its regulation by DNA damage remains to be studied.

In order to test if the inactivation of *MESR4*, *hephaestus*, and HLHm7 genes are responsible for the defective IR induced cell cycle arrest in the above three EPs, we determined the expression level of each gene by RT-PCR (Fig. 2C). The expression of *hephaestus* was undetectable in EP(3)3574 homozygous larvae, whereas the transcripts of *MESR4* and HLHm7 were detected in EP(2)0386 and EP(3)3587, respectively. These results suggest that inactivation of *hephaestus* might be responsible for the defects in EP(3)3574, while genes other than *MESR4* and HLHm7 could be the cause of defects in EP(2)0386 and EP(3)3587. Alternatively, the expression of *MESR4* and HLHm7 could be reduced in the EPs compared to the wild type. We did not perform quantitative PCR, nor could we distinguish the PCR products from cDNA and putative genomic DNA contamination in the RNA preparation due to lack of intron in HLHm7 gene. Thus, we cannot rule out the possibility that expression of *MESR4* and HLHm7 is attenuated in EP(2)0386 and EP(3)3587, which might result in incomplete penetrance of the phenotype. In order to confirm the genes that are responsible for the defective IR induced cell cycle arrest in the three EPs, generation of null mutants and genetic rescue by the particular gene will be necessary.

SUMMARY

In order to identify novel signaling components of Chk2 pathway, we performed a genetic screen of EP insertions for modifiers of a Chk2-dependent rough eye phenotype and identified 25 EP insertions affecting 20 genes. Some of these have known roles in gene regulation, spindle formation and neuronal dysfunction suggesting links between these processes and the DNA damage response. We also identified three EP insertions associated with defects in DNA damage-induced G2 arrest when homozygous. Further studies on these EPs will be needed to elucidate their underlying molecular defects, interaction with Chk2, regulation by DNA damage, and possible roles in tumorigenesis.

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