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</tr>
<tr>
<td><strong>Authors</strong></td>
<td>Philippe Krebs, Nengming Xiao, Bruce Beutler</td>
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<td><strong>Illustrators</strong></td>
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Phenotypic Description

The Endeka phenotype was discovered among ENU-induced G3 mutant mice in an in vivo natural killer (NK) cell and CD8+ cytotoxic T lymphocyte (CTL) cytotoxicity screen. G3 mice were immunized with irradiated 5E1 cells (syngeneic class I MHC-deficient cells transformed by human adenovirus type 5 early region 1). One week later, the same mice were injected with three target cell populations: control C57BL/6J cells, NK cell-specific target cells (syngeneic class I MHC-deficient cells), and an antigen-specific CTL target population (C57BL/6J splenocytes externally loaded with the adenovirus E1B protein). Both homozygous and heterozygous Endeka mice exhibit a reduced ability to kill class I MHC-deficient cells but kill antigen-specific targets normally, demonstrating impaired NK cell cytotoxicity but normal CD8+ CTL function (Figure 1A and 1B). The level of killing by heterozygous Endeka NK cells is intermediate between wild type and homozygous Endeka NK cells. Endeka homozygotes display a large reduction of NK1.1+ cells and CD8+ T cells in the blood (Figure 1C and 1D). Heterozygous animals display a reduction of

Figure 1. Impaired cytotoxic activity of Endeka NK cells. (A and B) The percentage of target cells killed by NK cells (A) or CD8+ T cells (B) from blood collected from mice of the indicated genotypes one week after immunization with 5E1 cells. (C and D) The percentage of NK cells (C) and CD8+ T cells (D) in blood from mice of the indicated genotypes. *P<0.05, **P<0.001, Student’s t test.

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NK1.1+ cells intermediate between wild type and homozygotes, while the number of CD8+ T cells is normal. Thus, with respect to the NK cell phenotype, Endeka is semidominant, but with respect to the CD8+ T cell phenotype, Endeka is recessive. Both heterozygous and homozygous animals have normal levels of CD4+ T cells, CD19+ B cells, and CD11c+ dendritic cells in their blood.

Peritoneal macrophages from homozygous Endeka mice produce reduced amounts of type I interferon (IFN) in response to lipopolysaccharide (LPS, TLR4 ligand) and poly I:C (TLR3 ligand). Tumor necrosis factor (TNF)-? production induced by LPS, poly I:C, CpG oligodeoxynucleotides (TLR9 ligand), resiquimod (TLR7 ligand), peptidoglycan (TLR2/6 ligand), macrophage-activating liopeptide-2 (MALP-2, TLR2/6 ligand), and Pam3CSK4 (TLR2/1 ligand) is normal in homozygous Endeka macrophages (TLR Signaling Screen). Heterozygous and homozygous Endeka mice display enhanced susceptibility to infection with 2 x 10^5 PFU of mouse cytomegalovirus (MCMV Susceptibility and Resistance Screen).

### Nature of Mutation

The Endeka mutation was mapped to Chromosome 11 on the recessive CD8+ T cell phenotype (reduced numbers of CD8+ T cells in blood), and corresponds to an A to G transition at position 345 of the Irf1 transcript, in exon 3 of 10 total exons.

329 GCTAAGCGGCTGGGACATCAACAAGGATGCC
42  -A--K--H--G--W--D--I--N--K--D--A-

The mutated nucleotide is indicated in red lettering, and causes an aspartic acid to glycine change at position 47 of the IRF1 protein.

### Protein Prediction

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In mice and humans, interferon regulatory factor (IRF)-1 is one of nine members of the IRF family of transcription factors, which regulate the transcription of type I interferons (IFN) and IFN-inducible genes during immune system development, homeostasis and activation by microbes. Mouse and human IRF1 exhibit 83% sequence identity (1). An additional IRF, IRF10, has been identified in chickens (2).

Mouse IRF1 contains 329 amino acids and functions as a transcriptional activator (Figure 2). As in the other IRFs, the N-terminal half of IRF1 (residues 1-113) serves as the DNA binding region, and is characterized by the presence of five tryptophans spaced ten to eighteen amino acids apart in a “tryptophan cluster” (residues 11, 26, 38, 58, and 77) (3). The DNA binding region bears similarity to that of the c-Myb oncoprotein that also contains a tryptophan cluster (4), but not to any other transcription factor classes. IRF family proteins share sequence and structural homology in their DNA binding regions, and all bind to a similar DNA motif (A/G NGAAANNGAAACT) called the IFN-stimulated response element (ISRE) (5) or IFN regulatory element (6), found within positive regulatory domain I and III (PRD I and PRD III) of the IFN-? promoter. By binding to the same element, the repressive IRF2 has been shown to antagonize transcription activated by IRF1 under certain circumstances (7). IRF1 forms a nucleoprotein complex with ATF2/c-Jun, NF-? B, and the high-mobility group protein HMG I(Y) to activate transcription of IFN-? (8). Interestingly, when combined in vitro, NF-? B and IRF1 cannot bind DNA simultaneously due to steric hindrance between the two proteins (3). The presence and binding of HMG I(Y) is required to relieve this hindrance and permit formation of the nucleoprotein complex (8).
Analysis of the crystal structure of the IRF1 DNA binding domain bound to the PRD I element reveals a cluster of three \( ? \)-helices (\( ? \)-1-\( ? \)-3) flanked on one side by a mixed four-stranded \( ? \)-sheet (\( ? \)-1-\( ? \)-4) \((3)\) (Figure 3, PDB ID 1IF1). Although topologically similar, the fold of the domain is distinct from that of the canonical DNA-binding helix-turn-helix (HTH) motif, in that the IRF1 DNA binding domain contains three large loops (LI, L2 and L3) connecting \( ? \)-2 to \( ? \)-2, \( ? \)-2 to \( ? \)-3, and \( ? \)-3 to \( ? \)-4, respectively. In addition, the mode of DNA interaction differs, with the \( ? \)-3 helix, or recognition helix, of IRF1 contacting the major groove of the DNA double helix at a 45° angle to the DNA axis. In contrast, in most HTH proteins, the recognition helix is oriented approximately perpendicular to the DNA axis \((9)\). The four nucleotides GAAA form the core sequence of the PRD I element recognized and contacted by the IRF1 recognition helix, although interactions exist up to six nucleotides away. Cooperative binding to DNA occurs when the GAAA sequence is repeated in tandem \((10)\). This cooperativity is induced by DNA structure distortion when the first IRF molecule binds, preparing a template for interaction with the next molecule. Arg 82, Cys 83, Asn 86 and Ser 87, which are located in the last two turns of the recognition helix, form contacts with GAAA. Loops L1 and L3 contact the DNA backbone, as do three of the five tryptophans of the tryptophan cluster (Trp 11, Trp 38, Trp 58). Binding of IRF1 to DNA induces a 22° bend from vertical in the DNA axis, narrowing the major groove in which the recognition helix is located.

By cellular localization studies of deletion constructs, the C-terminal half of IRF1 has been shown to contain a nuclear localization signal (amino acids 115-139) \((11)\). A transactivation domain is also reported to exist (amino acids 185-256) \((11)\). The C-terminal halves of all IRF family members contain either an IRF association domain 1 (IAD1) or an IAD2, with which they bind to IRF other family members, other transcription factors, or self-associate. These interactions allow the IRFs to modulate their activity and target a variety of genes. The IAD1 is approximately 177 amino acids in length, and is conserved in all IRFs except IRF1 and IRF2 \((12-14)\). IAD2 domains are found only in IRF1 and IRF2 \((13)\). In IRF1, the IAD2 has been mapped \textit{in vitro} using electrophoretic mobility shift (EMSA) and GST pull-down assays to two overlapping regions (amino acids 164-219 or 201-263) \((11;13)\). Finally, phosphorylation at several sites in the C-terminal half of IRF1 may modulate DNA binding and transcriptional activity; casein kinase II (CKII) may be one of the kinases to carry out this phosphorylation \((14;15)\).

The \textit{Endeka} mutation at position 47 of IRF1 exists within loop L1, which connects strand \( ? \)-2 to helix \( ? \). Aspartic acid 47 lies at the C-terminal end of loop L1, close to the boundary of helix \( ? \).
Irf1 mRNA is detected in most tissues by Northern blot, including heart, liver, lung, spleen, thymus, kidney, and intestine (1). In the thymus, IRF1 is expressed by mature CD4+ and CD8+ T cells, but not by CD4+CD8+ immature thymocytes (16). When overexpressed as a tagged protein in unstimulated cells, IRF1 is predominantly localized to the nucleus, with some expression in the cytoplasm (11;17).

Background

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IRF1 was the first discovered member of the IRF family of transcription factors, identified originally as a positive regulator of IFN-? gene transcription (1). In 1993, the generation of mice with a targeted deletion of IRF1 opened the door to discovery of the many processes controlled by IRF1. IRF1 regulates transcription in response to diverse signals received during development and homeostasis of the myeloid and lymphoid compartments, and upon activation of innate and adaptive immune receptors by microbial infection. In particular, IRF1 is required for the development and function of dendritic cells (DC), granulocytes, NK and NKT cells, CD4+ T cells, and CD8+ T cells (Figure 4). IRF1 functions in innate immune signaling from Toll-like receptor (TLR) 9 to activate a select group of genes. IRF1 also acts as a tumor suppressor, and its inactivation contributes to oncogenesis. These functions are reviewed below.

**DC development**

Multiple IRFs are involved in the regulation of DC development into the various DC subsets, which include the two major subsets, plasmacytoid DC (pDC) and conventional DC (cDC), and several cDC subsets distinguished by their positive or negative expression of CD8? and CD4 (18). Distinct functional specializations characterize each subset, and allow them to carry out their roles in defense against viruses and tumors, and maintenance of T cell tolerance. IRF1 has been reported to modulate the development and functional maturation of DC, and particularly, their tolerogenic ability. *Irf1*-/- mice have an increased population of pDC and a selective reduction of the CD8? subset of cDC (19). Splenic DC from *Irf1*-/- mice are impaired in their ability to produce proinflammatory cytokines such as IL-12, but express high levels of IL-10, TGF-? and the tolerogenic enzyme indoleamine 2,3 dioxygenase. *Irf1*-/- DC have a reduced ability to stimulate the proliferation of allogeneic T cells, and induce an IL-10-mediated suppressive activity in allogeneic CD4+CD25+ regulatory T cells. IRF1 thus appears to downregulate T cell tolerance by promoting production of proinflammatory cytokines while inhibiting production of anti-inflammatory cytokines by DC.

**Myeloid development**

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IRF1 mRNA is rapidly upregulated, followed later by IFN-? mRNA, upon induction in vitro of terminal differentiation of M1 myeloid precursor cells or 32Dcl3 myeloblasts (20;21). Ectopic expression of IRF1 can accelerate granulocytic differentiation stimulated by G-CSF (21). Thus, IRF1 induction may be a limiting step in the early events of differentiation. Consistent with this hypothesis, bone marrow from Irfl-/- mice contains an increased number of immature granulocytic precursors, and a decreased number of mature granulocytes compared to wild type bone marrow cells, as determined by cell staining for Gr-1 or CD11b (22). The colony-forming ability of Irfl-/- progenitors from the bone marrow in response to treatment with G-CSF and M-CSF is also reduced. These data support a role for IRF1 in promoting the maturation of granulocytes.

NK and NKT cell development
Irfl-/- mice exhibit a severe deficiency of NK cells, which lack in vitro cytotoxic activity in response to LCMV infection or poly I:C treatment in vivo and fail to produce IFN-? upon IL-12 stimulation in vitro (23-25). NKT and intestinal intraepithelial lymphocytes (IELs) are also greatly reduced in Irfl-/- mice (24). Interestingly, Irfl+/- mice display an intermediate reduction in NK, NKT and IEL numbers. These three lymphocyte subsets express the IL-15 receptor, and rely on IL-15 for their development and maintenance (26-28). Northern blot analysis demonstrates a failure of IL-15 induction in Irfl-/- bone marrow cells stimulated with LPS, suggesting that IRF1 regulates IL-15 gene expression (24;29). Indeed, the Il15 gene promoter contains a consensus ISRE sequence to which IRF1 binds and directly activates Il15 transcription (29). Interestingly, chimeras in which Irfl-/- bone marrow cells were transferred into irradiated wild type mice generated normal numbers of functional NK cells, while in contrast, chimeras containing wild type bone marrow cells transferred into irradiated Irfl-/- mice failed to generate any functional NK cells (29). Importantly, Irfl-/- bone marrow cells generate functional NK cells when cultured in the presence of exogenous IL-15. These data demonstrate that the deficiency of NK cells in Irfl-/- mice is not cell intrinsic, but is caused by a failure of bone marrow stromal cells that normally support NK cell development to produce IL-15.
Mutagenetix Phenotypic Mutation 'endeka'

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cells expressing ?? T-cell receptors (TCR) begin their development as double negative CD4-CD8- precursor thymocytes in the capsule of the thymus. After TCR? rearrangement, progression to the double positive CD4+CD8+ stage, and TCR? rearrangement (in the cortex of the thymus), the yet immature TCR?? +CD4+CD8+ thymocytes are then subject to positive or negative selection to generate mature CD4+ helper T cells and CD8+ cytotoxic T cells in the medulla of the thymus.

**T cell development and function**

CD4+ and CD8+ T cells expressing ?? T-cell receptors (TCR) begin their development as double negative CD4-CD8- precursor thymocytes (**Figure 5**). After TCR? rearrangement, progression to the double positive CD4+CD8+ stage, and TCR? rearrangement, the yet immature TCR?? +CD4+CD8+ thymocytes are then subject to positive or negative selection to generate mature CD4+ helper T cells and CD8+ cytotoxic T cells. Physical contacts between TCR/CD3 complexes and both costimulatory molecules on developing thymocytes and major histocompatibility (MHC) molecules on thymic stroma are important for T cell selection and commitment to the CD4+ or CD8+ lineages. The affinity and avidity of T cell-stromal cell interactions determines the outcome of thymic selection, with high affinity TCR-self-MHC interactions leading to elimination of the T cell (negative selection), and low affinity TCR-self-MHC interactions promoting T cell survival (positive selection). T cells expressing a TCR that does not recognize self-MHC molecules undergo receptor editing or die through apoptosis.

*Irfl*-/- mice display a 10-fold reduction of mature CD8+ T cells in the thymus, spleen, lymph nodes and blood, while CD4+ T cells are present in normal numbers (30;31). Analysis of immature thymocyte populations demonstrated a lineage-specific defect in maturation from the double-positive (CD4+CD8+) to single-positive (CD4+CD8+) stage during CD8+ T cell development. Thymic stromal cells and T cells at all stages of maturation were found to have reduced expression of MHC class I, which is secondary to a reduced expression of transporter associated with antigen processing-1 (TAP1) and the proteasome subunit low molecular mass protein 2 (LMP2) (16;32). Both TAP1 and LMP2 are required for presentation of peptides by MHC class I molecules, and both contain ISREs in their gene promoters that are transcriptionally activated by IRF1 (32). However, the defect in T cell maturation was found to be T cell-intrinsic rather than a consequence of a defective thymic stromal environment, as *Irfl*-/- stromal cells could support normal development of CD8+ thymocytes in bone marrow chimeras and in reaggregation cultures *in vitro* (16). Thus, impaired MHC class I expression on thymic stromal cells is at most only partially responsible for the maturation defect of *Irfl*-/- CD8+ T cells.

In addition to impaired MHC class I expression, *Irfl*-/- thymocytes have defective TCR-mediated signal transduction, which probably also contributes to the impaired positive and negative selection of CD8+ T cells. The induction of negative selection in TCR transgenic thymocytes from *Irfl*-/- mice required a 1000-fold higher level of selecting peptide than wild type thymocytes (16). Thus, IRF1 may regulate thymocyte gene expression important for lineage commitment and selection of CD8+ T cells. Transgenic overexpression of the anti-apoptotic protein Bcl2 under the E? or Lck promoter in *Irfl*-/- mice restores CD8+ T cell development (but not NK, NKT or TCR?? + intestinal intraepithelial lymphocyte development), suggesting that IRF1 is necessary for the propagation of survival signals from Bcl2 (33).
IRF1 is differentially required for control of various viral infections. For example, when infected in vivo with LCMV, the cytotoxic response against infected target cells is significantly reduced in Irf1\(^{-/-}\) mice, likely due to their dramatically reduced numbers of CD8\(^{+}\) T cells (30). However, viral titers from LCMV-infected Irf1\(^{-/-}\) mice were similar to those of wild type mice three days after infection, and mutants effectively cleared the virus by six days after infection, indicating that IRF1 is not required for cytotoxic activity of CD8\(^{+}\) T cells, and consequently, for controlling LCMV. In contrast, IRF1 is required for control of infection by encephalomyocarditis virus (EMCV) and coxsackievirus B3 (34). In these cases, IRF1 contributes to the establishment of the IFN-induced antiviral state.

Once committed to the CD4\(^{+}\) lineage, CD4\(^{+}\) T cells can be stimulated to differentiate into type 1 T helper (Th1) or type 2 T helper (Th2) cells, as well as other Th types. The differentiation of CD4\(^{+}\) T cells into Th1 or Th2 cells, controlled largely by IL-12 and IL-4, respectively, determines the nature of the immune response to infections. Th1 and Th2 cells produce distinct sets of cytokines. Th1-type cytokines are generally proinflammatory and include IFN-\(\gamma\), IL-2 and TNF-\(\alpha\); Th2-type cytokines are generally anti-inflammatory and include IL-4, IL-5, IL-10, and IL-13. Th2 cells are thought to promote allergic responses. Although Irf1\(^{-/-}\) CD4\(^{+}\) T cells mature normally, they exclusively undergo Th2 differentiation (25;35). This is manifested by a skewed profile of cytokine production (dominated by IL-4 and lacking IFN-\(\gamma\)) when Irf1\(^{-/-}\) mice are infected with Leishmania major in vivo and when Irf1\(^{-/-}\) splenic cells are stimulated with TCR-specific peptide in vitro (25;35). The failure of Th1 differentiation is due in part to the inability of Irf1\(^{-/-}\) mice to both produce and respond to IL-12. Irf1\(^{-/-}\) DC and macrophages exhibit impaired production of IL-12 (17;19;25). IRF1 is itself induced by IL-12 (36;37), and Irf1 activates transcription of both IL-12p40 and IL-12R\(^{\beta2}\) through direct binding to their gene promoters (38;39). The lack of NK cells, which normally produce IFN-\(\gamma\), to stimulate macrophages to secrete IL-12, further compromises Th1 differentiation in Irf1\(^{-/-}\) mice.

**IRF1 and TLR signal transduction**

IRFs are now known to be critical components of innate immune signaling pathways. IRF3, IRF5, and IRF7 are particularly important, functioning in MyD88-dependent and -independent TLR signaling, as well as in RIG-I/MDA5 signaling [reviewed in (40)]. IRF1 has been shown to directly interact with MyD88 in both FRET and co-immunoprecipitation assays (17). Through an unknown mechanism, this interaction is thought to promote the efficient translocation of IRF1 to the nucleus, where it activates the transcription of selected genes, including those encoding IFN-\(\gamma\), inducible nitric oxide synthase (iNOS), and IL-12p35. IRF1 has been proposed to mediate the synergistic effect of IFN-\(\gamma\) and TLR signaling on gene transcription, a hypothesis supported by the findings that IRF1 mRNA is strongly induced by IFN-\(\gamma\), and that IRF1 is required for the induction of IFN-\(\gamma\) mRNA by CpG-A (a TLR9 ligand) in bone marrow-derived cDC. IFN-\(\gamma\) induction by poly I:C or LPS (TLR3 and TLR4 ligands, respectively) is normal in Irf1\(^{-/-}\) bone marrow-derived cDC, indicating that TLRs differentially utilize IRF1 to mediate their effects. TNF-induced IFN-\(\gamma\) production by macrophages also depends on IRF1, and initiates an autocrine loop that sustains expression of inflammatory genes (41).

**IRF1 in tumor suppression**

The induction of apoptosis is a mechanism by which pre-cancerous cells are eliminated, and mutations in tumor suppressor genes can prevent this process. Expression of an activated c-Ha-ras oncogene is sufficient to transform Irf1\(^{-/-}\) but not wild type fibroblasts, and Irf1\(^{-/-}\) fibroblasts fail to undergo apoptosis when treated with ionizing radiation and transformed by c-Ha-ras, demonstrating that IRF1 functions as a tumor suppressor (42). In mice, although inactivation of IRF1 does not cause spontaneous tumor development, lack of IRF1 exacerbates tumor...
predisposition caused by transgenic expression of c-Ha-ras or homozygous null mutations of p53 (43). DNA damage-induced cell cycle arrest is also defective in Irf1−/− fibroblasts, due to a failure to transcriptionally activate the cell cycle inhibitor p21/CIP1/WAF1 (44). IRF1 acts together with p53 in the regulation of p21 transcription. Interestingly, DNA damage-induced apoptosis depends on IRF1, but not p53, in mature T lymphocytes; p53, but not IRF1, is required for apoptosis in thymocytes (45,46).

Human IRF1 is located on chromosome 5q31.1 (47), within a region frequently deleted in human leukemias and the preleukemic myelodysplastic syndromes (MDS, OMIM #153550) (48). Of the genes in the 5q31.1 region, only IRF1 was consistently deleted at one or both alleles in thirteen cases of leukemia or myelodysplasia associated with 5q31 abnormalities, providing evidence that IRF1 is the tumor suppressor mutated in these diseases (49). In another study, twelve of fourteen patients with 5q deletions and acute myeloid leukemia or MDS had loss of one allele of IRF1 (50). Loss of an IRF1 allele has also been reported to occur in gastric and esophageal cancers (51,52).

Transcriptional targets of IRF1
The targets of IRF1 continue to be investigated. IRF1 is reported to bind to ISREs in many IFN-inducible gene promoters, such as those for inducible nitric oxide synthase (iNOS) (53), cyclooxygenase-2 (Cox-2) (54), class II transactivator (CIITA) (55), and guanylate-binding protein (GBP) in IFN-stimulated macrophages (34). Transcription of IRF1 itself is also induced by IFN-α (56). Downstream of TLR9, IRF1 induces IL-12p40, IL-12p40, iNOS, IL-18, and IFN-α (17,55). The target genes of IRF1 responsible for apoptotic responses may include genes encoding Caspase 1 (45), Caspase 7 (57), and TNF-related apoptosis-inducing ligand (TRAIL) (58).
Although not all of the same phenotypes have been examined, $Irf1^{Endeka}$ mice display essentially identical defects to those of $Irf1^{-/-}$ mice. As observed for the $Endeka$ allele, the reduction of CD8$^+$ T cells is a recessive phenotype caused by homozygosity for the null allele of $Irf1$. The defect in CD8$^+$ T cell development is cell-intrinsic, and heterozygous thymocytes must have enough IRF1 function to activate the genes essential for their lineage commitment and selection. In contrast, the reduction of NK cells is a semidominant phenotype. NK cells are reduced because of a cell-extrinsic defect in which bone marrow stromal cells fail to produce the IL-15 required for NK cell development and maintenance. IL-15 is a membrane-associated molecule that is presented in trans by antigen presenting cells to cells expressing the IL-15 receptor, such as NK and CD8$^+$ T cells. Treatment with soluble recombinant IL-15 stimulates NK cell proliferation in a dose-dependent manner (26). Thus, in mice with heterozygous IRF1 mutations, it may be predicted that stromal cells express half of the normal levels of IL-15 on their surface, an amount sufficient to engage and stimulate reduced numbers of NK cells to survive.

The similarity between phenotypes of IRF1 null and $Endeka$ mice suggests that the $Endeka$ mutation completely abolishes IRF1 activity and/or expression. The position of Asp 47 close to Trp 38, Ala 41, Lys 43, and His 44, all of which lie within loop L1 and make hydrogen bonds with the phosphate backbone, suggests that mutation of Asp 47 may disrupt the interactions of loop L1 with DNA. Asp 47 exists adjacent to a turn beginning with Ile 48 in the three dimensional structure of IRF1, between bulky and nonpolar Trp and Ile residues. Mutation to Gly may disrupt the structure of loop L1. Asp 47 itself has not been found to make direct contacts with DNA. His 40 is also predicted to form a contact with the DNA via a hydrogen bond with a water molecule that bridges the distance between protein and DNA, as observed in the crystal structure of the IRF2 DNA binding domain (10). This interaction may also be disrupted by the $Endeka$ mutation. Analysis of the DNA binding properties of the protein encoded by $Irf1^{Endeka}$ may reveal critical function(s) for Asp 47 in the IRF1-DNA interaction.

**Genotyping**

$Endeka$ genotyping is typically performed by phenotypic analysis of mice (measurement of CD8$^+$ T cell and NK cell numbers in blood). PCR and DNA sequencing is used for genotyping in the following protocol, which has not been tested. The region containing the mutation is amplified using PCR, followed by sequencing of the amplified region to detect the single nucleotide transition.

**Primers**

$Endeka$ (F): 5'- TTGGGAGTATGAGCAGGAGCCTATC -3'
$Endeka$ (R): 5'- TAACTGCGCTGACTTGGACCTCAAC -3'

**PCR program**

1. 95°C  2:00
2. 95°C  0:30
3. 56°C  0:30
4. 72°C  1:00
5. repeat steps (2-4) 29X
6. 72°C  7:00

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7) 4°C

Primers for sequencing
Endeka_seq(F): 5’- TGTGGCTCATCAGAACCTAAG -3’
Endeka_seq(R): 5’- CACCCAGAACACTGATGGTA -3’

The following sequence of 1022 nucleotides (from Genbank genomic region NC_000077 for linear genomic sequence of Irfl) is amplified:

2358  ttg ggagtatgag caggagccta tc tatgttagg agtcactgtg
2401  ccaagcgctc tggagagact tc tgaagcaag aaggtcccat gttccaatgc tcggtagcca
2461  cagcgacagca tgcgggggta aaaggggaa gtcagccttt tcccatatct
2501  aggagtcgct catccteggt cpacactcga gcgtactcgg tctctgtatg acacgttca
2561  gagaagag atctccacga tccagtgaag ccggagcagc gcctgagaag ggggtgcctc ttaggttcctc
2621  agacatcctc cagcgccgta tgcgggggta aaaggggaa gtcagccttt tcccatatct
2671  gggggtgcg cagagaggcc tgtgcgggta aaaaggggaa gtcagccttt tcccatatct
2731  cagcgacagca tgcgggggta aaaggggaa gtcagccttt tcccatatct
2791  agacatcctc cagcgccgta tgcgggggta aaaggggaa gtcagccttt tcccatatct
2851  gggggtgcg cagagaggcc tgtgcgggta aaaaggggaa gtcagccttt tcccatatct
2911  cagcgacagca tgcgggggta aaaggggaa gtcagccttt tcccatatct
2971  gggggtgcg cagagaggcc tgtgcgggta aaaaggggaa gtcagccttt tcccatatct
3031  cagcgacagca tgcgggggta aaaggggaa gtcagccttt tcccatatct
3091  gggggtgcg cagagaggcc tgtgcgggta aaaaggggaa gtcagccttt tcccatatct
3151  cagcgacagca tgcgggggta aaaggggaa gtcagccttt tcccatatct
3211  gggggtgcg cagagaggcc tgtgcgggta aaaaggggaa gtcagccttt tcccatatct
3271  gggggtgcg cagagaggcc tgtgcgggta aaaaggggaa gtcagccttt tcccatatct
3331  gggggtgcg cagagaggcc tgtgcgggta aaaaggggaa gtcagccttt tcccatatct

Primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated A is highlighted in red.

References


Cite this information as follows: Philippe Krebs, Nengming Xiao, Eva Marie Y. Moresco, Beutler B. Record for endeka, updated Dec 17, 2018. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu
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