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Letter to the Editor

Delta and *Notch* promote correct localization of IrreC-rst

Dear Editor,

The *Notch* signal transduction pathway has been implicated in a variety of cell fate decisions throughout development. In the developing *Drosophila* retina, the Notch receptor and its ligand Delta are required to organize the interommatidial lattice through selective programmed cell death (PCD).[1,2] Another transmembrane molecule required for PCD in the *Drosophila* retina is the immunoglobulin superfamily member Irregular chiasmC-roughest (IrreC-rst).[3–5] Loss-of-function mutations in *irreC-rst* lead to a block in PCD during retinal development, resulting in a rough eye phenotype in the adult. In addition, the *irreC-rst*^{CT} mutation, which results in truncation of the IrreC-rst intracellular domain, is associated with reduced IrreC-rst protein at the plasma membrane and increased IrreC-rst within intracellular vesicles.[4,5] Retinal PCD is lost, indicating that proper subcellular localization of IrreC-rst may be an important factor in permitting or promoting the death process. The mechanisms by which Notch signaling and IrreC-rst regulate PCD are unclear.

In a recent genetic screen (Tanenbaum *et al*, submitted) and in direct tests, *Delta* (*Dl*) mutations were identified as dominant enhancers of the rough eye phenotype conferred by the hypomorphic allele *irreC-rst*^{3} (n=7 *Dl* mutant lines; Figure 1A-C). These observations demonstrate that *Delta* and *irreC-rst* interact genetically to execute PCD, and motivated us to examine further the relationship between Delta/Notch signaling and IrreC-rst.

In a wild-type retina, differentiation of the primary pigment cells (1°s) in the young pupa is followed by selective removal of approximately one-third of the neighboring interommatidial precursor cells by PCD.[3,6] The remaining interommatidial precursor cells are organized concurrently into a hexagonal array and differentiate as optically-insulating secondary/tertiary pigment cells (2°/3°s; Figure 1D). During the state of PCD, IrreC-rst protein is expressed at high levels in the 2°/3°s and at low levels in the 1°s. It is localized subcellularly along the border between the 2°/3°s and the 1°s, and between the two 1°s of each ommatidium[5] (Figure 1D-F). This subcellular localization of IrreC-rst is lost in two *Notch* alleles ($N^{fa\text{-}g2}$, $N^{fa\text{-}swb}$) that reduce *Notch* activity specifically in the young pupal retina during pigment cell differentiation; however, these alleles also result in a loss of 1°s and a block in PCD.[1,5] Mislocalization of IrreC-rst could represent an indirect effect due to defects in 1° differentiation or PCD. Alternatively, Notch protein or signaling could affect more directly the localization of IrreC-rst.

To distinguish between these possibilities, we utilized temperature-sensitive alleles to reduce *Delta* and *Notch* function specifically during the stage of PCD, subsequent to formation of the 1°s.[1,2,7] Flies heterozygous for the temperature sensitive allelic combination Dl^{RF}/Dl^{6B} were shifted to the non-permissive temperature. A temperature shift of 6 h duration resulted in a loss of IrreC-rst localization: the IrreC-rst protein was redistributed throughout the apical surfaces, revealing the boundaries between 2°s and 3°s (Figure 1G). Similarly, shifting N^{ts1} pupae to the non-permissive temperature for 8 h resulted in a redistribution of IrreC-rst protein (Figure 1H). In parallel control experiments, redistribution of IrreC-rst protein was not observed in temperature shift experiments with wild-type or with either Dl^{RF} or Dl^{6B} *in trans* to a normal copy of *Delta* (Figure 1E,F), in Dl^{RF}/Dl^{6B} flies shifted for intervals ranging from 0 to 4 h, or in unshifted N^{ts1} flies (data not shown).

The observed redistribution of the IrreC-rst protein was not an indirect effect of a loss of PCD in the Dl^{RF}/Dl^{6B} and N^{ts1} backgrounds: when PCD was blocked by directed expression of the activated dRas1 isoform dRas1^{val12} [8,9] or the baculovirus caspase inhibitor p35,[10] IrreC-rst localization was unaffected (Figure 1I,J). This indicates that the redistribution of IrreC-rst protein is a specific effect of the reduction of *Notch* or *Delta* activity or protein and not a result of incorrect 2°/3° patterning. Correct localization of IrreC-rst is likely important for its normal function,[5] and it will be interesting to determine the mechanism by which it is regulated through *Delta* and *Notch*.

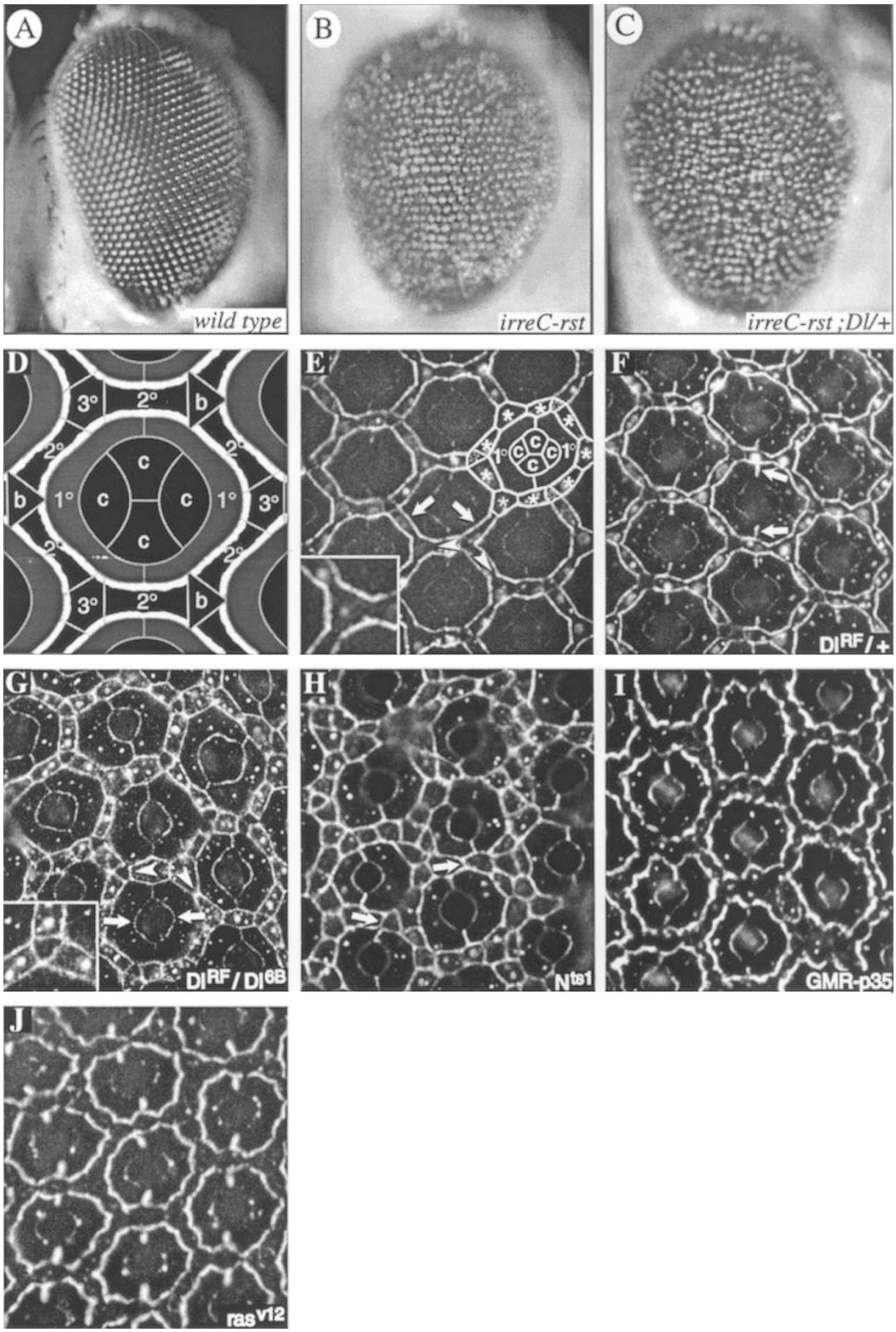

Figure 1 The rough eye phenotype of *irreC-rst*^{3} is enhanced by Delta^{E58}. (**A**) A wild-type adult eye contains approximately 750 ommatidia arranged in straight lines along multiple axes. (**B**) A *y irreC-rst*^{3}; *dp*^{ov}; *p*^{p} adult eye has a mildly altered pattern of ommatidia compared to wild-type; careful inspection reveals an occasional 'jog' in the ommatidial rows. (**C**) These alterations are enhanced significantly in an *irreC-rst*^{3}; *Delta*^{E58}/+ adult eye. Six other *Dl* alleles were identified as dominant enhancers of *irreC-rst*^{3}: *Dl*^{J17}, *Dl*^{J111}, *Dl*^{K75} (Tanenbaum *et al*, submitted), *Dl*^{HD82}, *Dl*^{S130403} and *Dl*^{S148504}. In addition, *Dl*^{E58}, *Dl*^{J17}, *Dl*^{K75}, *Dl*^{J111} and *Dl*^{HD82} were observed to enhance *irreC-rst*^{UB883} and *irreC-rst*^{IR34}; the other alleles were not tested. (**D-J**) Localization of IrreC-rst protein in pupal retinae. In (**E**–**H** and **J**)

flies received a heat shock (see below), were dissected immediately, and immunostained using the IrreC-rst-specific antibody mAb 24A5.1.[11] (**D**) Schematic of the apical surface of a wild-type pupal retina. One central ommatidium is shown, and is labeled to indicate the non-neuronal cone cells (c), the optically-insulating primary pigment cells (1°), the optically-insulating secondary (2°) and tertiary (3°) pigment cells, and bristles (b). The photoreceptor core consisting of eight neurons is not visible in this view. Subcellular localization of IrreC-rst protein in 2°/3°s is indicated by white shading. For clarity, IrreC-rst expression in 1°s is omitted. (**E**) Wild-type control retina from an animal aged until 44 h after puparium formation (APF; 18°C), shifted to 32°C for 6 h and dissected immediately. One ommatidium and its associated 2°/3° lattice is traced (compare with (**D**)); the cone cells (c), 1°s, 2°/3°s (white asterisks), and bristles (filled asterisks) are labeled. Note the distinct localization of IrreC-rst protein between 1°s and 2°/3°s (e.g., arrows) but not, for example, between two 2°/3°s cells (arrowheads). An enlarged view of a single central 2°/3°s and three 2°/3°s radiating from it are shown in the inset; the boundaries between 2°/3°s cannot be seen due to a lack of IrreC-rst protein (compare with (**G**)). The vesicles within the 2°/3°s are likely multivesicular bodies, sites of protein internalization and turnover.[12] (**F**) Dl^{RF}/+ control retina. A 6 h heat pulse, performed as in (**E**), had no effect on IrreC-rst localization. Subcellular localization between two 1°s is more obvious than in (**E**) (arrows). (**G**) Dl^{RF}/Dl^{6B} retina following a 6 h heat pulse as in (**E**). IrreC-rst protein is no longer localized primarily at the border between 1°s and 2°/3°s but is distributed equally throughout the 2°/3° cells. This can be seen most easily with the staining between two 2°/3°s (e.g., arrowheads). The inset contains an enlarged view of a central 2°/3°; its boundaries with three neighboring 2°/3°s can now easily be seen due to re-localization of IrreC-rst (compare with (**E**)). The 1° membranes that directly abut cone cells (arrows) also appear to have increased levels of IrreC-rst staining at the expense of regions that contact a neighboring 1° (compare with arrows in (**F**)). (**H**) N^{ts1} retinae that received an 8 h heat pulse demonstrated a similar redistribution of IrreC-rst as Dl^{RF}/Dl^{6B} retinae; arrows indicate two examples. The additional 2°/3° cells – the result of a block in PCD – are particularly evident in this retina; they can be recognized as 2°/3°s based on their small apical profile and their position between ommatidia (see also[1]). $Notch^{ts1}$ pupae were aged until 42 h APF at 18°C or 21 h APF at 25°C prior to the 8 h, 32°C heat pulse. (**I**) Expression of the caspase inhibitor p35 throughout the retina leads to a loss of programmed cell death, but had no effect on the localization of IrreC-rst protein. Shown is a retina from *GMR-p35* at 28 h APF (25°C). (**J**) Retina from *hs-dRas1*^{val12}*/Cy0* following a 1 h heat shock (37°C) at 26 h APF (25°C) and dissected immediately. Localization of IrreC-rst is similar to wild-type

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SM Gorski[1], C Baker Brachmann[1], SB Tanenbaum[1] and RL Cagan[*,1]*

[1]Department of Molecular Biology and Pharmacology, Washington University School of Medicine, Campus Box 8103; 660 South Euclid Avenue, St. Louis, Missouri 63110, USA
*Corresponding author: E-mail: cagan@molecool.wustl.edu

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