Drosophila p53 controls Notch expression and balances apoptosis and proliferation

Short title: Dp53 controls Notch expression

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Rocío Simón¹, Ricardo Aparicio¹, Ben E. Housden², Sarah Bray² and Ana Busturia¹*

*corresponding author: abusturia@cbm.csic.es

office: +34 911 964 689
laboratory:+34 911 964 690
Fax: +34 911 964 420

Address
¹Centro de Biología Molecular “Severo Ochoa” CSIC-UAM.
c) Nicolás Cabrera 1, 28049 Madrid, Spain
²Department of Physiology Development and Neuroscience
University of Cambridge
Downing Street, Cambridge, CB2 3DY, UK
Supplementary Fig. S1. The Notch [2.7-NRE] element and conservation of putative consensus Dp33 binding sequences across Notch Drosophila genes. (a) Notch genomic region with graph showing Su(H) ChIP enriched regions in chromatin from wing imaginal discs (green; fold enrichment relative to total input -0.5-2.1, log2 scale; [1]). Matches to Su(H) binding motif are indicated (blue bars height indicates match to Patser position weigh matrix; [2]). Gene models are depicted in black. Orange coloured bar represents the region (2.7NRE) cloned to generate Notch[2.7-NRE]-GFP. (b) Notch[2.7-NRE] directs expression in wing imaginal discs, high-levels of GFP expression are detected at the dorsal-ventral boundary, a known site of Notch activity. (c) Notch[2.7-NRE]-GFP responds to ectopic Notch activity in ptc-Gal4/+; Notch[2.7-NRE]-GFP; UAS-Notch-intra (NICD)/+ wing disc. ptc-Gal4 driven expression of NICD in a stripe along the anterior-posterior boundary results in ectopic stripe of Notch[2.7-NRE]-GFP expression (arrow). (d) Location of the two Dp33 putative binding sites in the Notch[2.7-NRE] element (indicated as red triangles). (e-f) Conservation of the putative Dp33 binding sites (e, site 1; f, site 2) (light blue squares) located in the different Notch genes of the indicated Drosophila species (http://genome.ucsc.edu/).
Supplementary Fig. S2. Effects of Dp53 levels of expression on Notch and PH3 expression. (a-b) Low levels of Dp53 do not affect wing morphology. (a) Wild type wing, (b) Dp53\textsuperscript{5A-1/4}/Dp53\textsuperscript{5A-1/4} homozygous mutant wing. Note that both wings are similar, if not identical. Scale bars represent 200\,\mu m. (c-d) Inactivation of Dp53 does not affect NICD expression levels. (c) ptcGal4/+; UAS-GFP/+ wing imaginal disc showing patched-Gal4 domain of expression (green), (d) ptcGal4/+; UAS-Dp53\textsubscript{RNAi}/+ wing imaginal disc showing NICD expression (red). Note that NICD expression is not altered in the cells where Dp53 has been inactivated. Left inset shows a magnification of the indicated area (white square). Scale bars represent 50\,\mu m. (e-f) Efficiency of the UAS-Dp53RNAi line. (e) sd-Gal4/+; UAS-DΔNp53/+; UAS-GFP/+ wing, (f) sd-Gal4/+; UAS-DΔNp53/+; UAS-Dp53\textsubscript{RNAi}/+ wing. Note that the Dp53-overexpressing wing phenotype is almost completely rescued when Dp53 is simultaneously inactivated showing the efficiency of the UAS-Dp53\textsubscript{RNAi} line. Scale bars represent 200\,\mu m. (g-h) High levels of Dp53 in the wing pouch region of the discs do not affect the levels of PH3 expression in the notum region of the discs. (g) sd-Gal4/+ wing imaginal disc stained with anti-PH3 antibody (red), (h) sd-Gal4/+; UAS-DΔNp53/+ wing imaginal disc stained with anti-PH3 antibody (red). Note that while the amount of PH3 expressing cells is significantly increased in the wing pouch region (see also Figure 3) due to the protruding overgrowths, the number of PH3 expressing cells in the notum region (arrows) seem not to vary. Scale bars represent 50\,\mu m.
Supplementary Fig. S3. Effects of the inhibition of apoptosis and inactivation of *Notch* on the C3 and NICD expression. (a-b) Low levels of *Notch* do not induce C3 expression. (a) Wing pouch region of a *sd-Gal4/+; UAS-Notch<sub>RNAi</sub>/+* wing imaginal disc showing C3 expression (red), (b) wing pouch region of a *N<sup>55e11</sup>/+* wing imaginal disc showing C3 expression (red). (c-d) Low levels of *Notch* and *Dp53* do not induce C3 expression. (c) Wing pouch region of a *N<sup>55e11</sup>/+; Dp53<sup>5A-1-4</sup>/+* wing imaginal disc showing C3 expression (red), (d) wing pouch region of a *N<sup>55e11</sup>/+; Dp53<sup>5A-1-4</sup>/Dp53<sup>5A-1-4</sup>* wing imaginal disc showing C3 expression (red). (e-f) High levels of DIAP1 inhibit C3 expression. (e) Wing pouch region of a *sd-Gal4/+; UAS-DΔNp53/+; UAS-GFP/+* wing imaginal disc showing C3 expression (red), (f) wing pouch region of a *sd-Gal4/+; UAS-DΔNp53/UAS-DIAP1* wing imaginal disc showing C3 expression (red). (g-h) Inhibition of apoptosis does not up-regulate NICD expression. (g-g<sup>+</sup>) Wing pouch region of a *sd-Gal4/+; UAS-DIAP1/+; UAS-GFP/+* wing imaginal disc showing (g) NICD expression (red) and (g<sup>+</sup>) GFP expression (green) that indicates the *sd-Gal4* domain. (h-h<sup>+</sup>) Wing pouch region of a *sd-Gal4/+; UAS-GFP/UAS-p35* wing imaginal disc showing (h) NICD expression (red) and (h<sup>+</sup>) GFP expression (green) that indicates the *sd-Gal4* domain. Scale bars represent 50μm.
Supplementary Fig. S4. Genetic interactions between Dp53 and wingless. The genetic interaction between Dp53 and wingless (wg) was determined by the analysis of the penetrance and severity of the transformation of the wing to notum produced in mutant wg flies [3]. (a) Graph representing the penetrance and severity of Dp53 and wg interaction in the wing. Penetrance is given as the percentage of flies showing a phenotype of the indicated severity. Severity is represented by a color code: wild type in blue, one wing transformed to notum in red, and two wings transformed in green. The number of flies studied in each case (1-4, indicated genotype) were n=156, n=100, n=65 and n=96 respectively.
Supplementary Materials and Methods

DNA sequence analysis. Identification of putative Dp53 binding sites in Notch gene sequence was performed with the assistance of the Bioinformatics Platform of the Universidad Autónoma de Barcelona. The canonical p53 consensus response element (p53RE) is defined as one or two tandem copies of the decamer motif “RRRCWWGYYY” separated by a 0-13 base pair spacer (core motif is highlighted in bold) where “R” represents purines, “W” represents adenine or thymine, and “Y” represents pyrimidines [4]. The different p53 weight matrices with the two highest hits are available upon request.

Supplementary references


