Local overexpression of Su(H)-MAPK variants affects Notch target gene expression and adult phenotypes in Drosophila

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Keywords: Drosophila, EGFR signalling, MAPK, Notch signalling, Su(H)

Abstract

In Drosophila, Notch and EGFR signalling pathways are closely intertwined. Their relationship is mostly antagonistic, and may in part be based on the phosphorylation of the Notch signal transducer Suppressor of Hairless [Su(H)] by MAPK. Su(H) is a transcription factor that together with several cofactors regulates the expression of Notch target genes.

Here we address the consequences of a local induction of three Su(H) variants on Notch target gene expression. To this end, wild-type Su(H), a phospho-deficient Su(H)MAPK-ko and a phospho-mimetic Su(H)MAPK-ac isoform were overexpressed in the central domain of the wing anlagen. The expression of the Notch target genes cut, wingless, E(spl)m8-HLH and vestigial, was monitored. For the latter two, reporter genes were used (E(spl)m8-lacZ, vgBE-lacZ). In general, Su(H)MAPK-ko induced a stronger response than wild-type Su(H), whereas the response to Su(H)MAPK-ac was very weak. Notch target genes cut, wingless and vgBE-lacZ were ectopically activated, whereas E(spl)m8-lacZ was repressed by overexpression of Su(H) proteins. In addition, in epistasis experiments an activated form of the EGF-receptor (DERact) or the MAPK (rSEM) and individual Su(H) variants were co-overexpressed locally, to compare the resultant phenotypes in adult flies (thorax, wings and eyes) as well as to assay the response of the Notch target gene cut in cell clones.

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### Value of the data

- This data shows the responses of several Notch target genes to modulations of Su(H) activity by the EGFR pathway.
- The data allow for a visual comparison of the spectrum of Notch target gene responses to Su(H) overexpression.
- Overexpression of activated components of the EGFR pathway and Su(H) variants, alone or in combination, can be compared in various Drosophila tissues.
- This data may be extended by analyses on DER<sup>act</sup> activity during Drosophila wing development.

### 1. Data

Suppressor of Hairless [Su(H)] is the transcription factor that regulates the expression of the target genes of the Notch signalling pathway [1,2]. Su(H) protein may be phosphorylated by MAPK as a result of Epidermal Growth Factor Receptor (EGFR) activation, providing a means of a direct cross-talk between these two pathways [3-5]. The response of several Notch target genes to the modulations of Su(H) by EGFR signalling activity was analysed by the local overexpression of either wild-type Su(H), a phospho-deficient Su(H)<sup>MAPK-ko</sup> and a phospho-mimetic Su(H)<sup>MAPK-ac</sup> variant [3] using the Gal4::UAS system [6], and staining of the tissues with respective antibodies. Moreover, activated components of the EGFR pathway (DER<sup>act</sup>, ri<sup>SEM</sup>) were overexpressed alone or in combination with individual Su(H) variants. The response of the Notch target gene cut was observed in cell clones of wing imaginal discs, and the resultant phenotypes on thorax, wings and eyes were recorded in adult flies.

#### 1.1. Overexpression of Su(H) variants during wing development

UAS-Su(H), UAS-Su(H)<sup>MAPK-ko</sup> and UAS-Su(H)<sup>MAPK-ac</sup> were overexpressed with omb-Gal4 [7] in wing imaginal discs of third instar Drosophila larvae. A total of four Notch target genes was analysed, wingless (Fig. 1) [8], cut (Fig. 2) [9], E(spl)m8-HLH [10] (using E(spl)m8-lacZ [11], Fig. 3) and vestigial [12] (using vg<sup>RE</sup>-lacZ [13], Fig. 4). Overall, overexpression of Su(H)<sup>MAPK-ko</sup> caused a stronger response of the Notch target genes than that of wild-type Su(H), whereas Su(H)<sup>MAPK-ac</sup> elicited the weakest effects, in agreement with a downregulation of Su(H) activity by MAPK-mediated phosphorylation [3].
1.2. Response of cut expression to the combined induction of Su(H) variants and activated components of the EGFR pathway during wing development

The expression of the Notch target gene cut was analysed in cell clones overexpressing either of the three Su(H) isoforms alone or in combination with the activated EGF-receptor (DER\textsuperscript{act}) or the activated MAPK (\textit{r}SE\textsuperscript{SEM})\textsuperscript{14,15} (Fig. 5). Overexpression clones were induced in wing imaginal discs \textsuperscript{16}. Su(H) overexpression induced cut expression, whereas it repressed it when simultaneously

|        | omb-Gal4 X | anti-Wg  | merge |
|--------|------------|----------|-------|
| UAS-LacZ |            |          |       |
| UAS-Su(H) |            |          |       |
| UAS-Su(H)\textsuperscript{MAPK-ko} |            |          |       |
| UAS-Su(H)\textsuperscript{MAPK-ac} |            |          |       |

\textbf{Fig. 1.} Response of the Notch target gene \textit{wingless}. Overexpression of the UAS-Su(H) variants as indicated; the \textit{omb}-expression domain is highlighted in blue in A–D and A′–D′ (A.A′ anti-beta galactosidase staining; B–D and B′–D′, anti-Su(H) staining). Expression of \textit{wingless} (Wg) is shown in red (A–D′). UAS-lacZ served as control. Note expansion of \textit{wingless} expression along the dorso-ventral boundary (arrows) upon overexpression of Su(H) and Su(H)\textsuperscript{MAPK-ko}, but not Su(H)\textsuperscript{MAPK-ac}. Overgrowth of the ventral disc is marked by asterisks and is a consequence of the overexpression of Su(H) protein (B′–D′).
overexpressed with \textit{rlSEM} (Fig. 5A–A‴ and C–C‴) [3]. Likewise repression was observed with \textit{Su(H)}\textsuperscript{MAPK-ko}, but less with \textit{Su(H)}\textsuperscript{MAPK-ac} (Fig. 5D and E‴). Cell clones overexpressing DER\textsuperscript{act} were frequently distorted, and \textit{cut} expression was induced at the boundary of DER\textsuperscript{act} expressing and non-expressing cells independent of the overexpression of any \textit{Su(H)} variant (arrowheads in Fig. 5F–I‴).

Fig. 2. Response of the Notch target gene \textit{cut}. Overexpression of the UAS-\textit{Su(H)} variants as indicated; the \textit{omb}-expression domain is highlighted in blue in A–D and A′–D′ (A,A′ anti-beta galactosidase staining; B–D and B′–D′, anti-Su(H) staining). Expression of \textit{cut} is shown in red (A–D′). UAS-lacZ served as control. Note expansion of \textit{cut} expression along the dorso-ventral boundary (arrows) upon overexpression of \textit{Su(H)} and \textit{Su(H)}\textsuperscript{MAPK-ko}, but not \textit{Su(H)}\textsuperscript{MAPK-ac}. Overgrowth of the ventral disc is marked by asterisks and is a consequence of the overexpression of \textit{Su(H)} protein (B′–D′).
1.3. Adult phenotypes resulting from the combined overexpression of Su(H) variants and activated components of the EGFR pathway

Overexpression of UAS-deract in the thorax (Fig. 6) or the wing anlagen (Fig. 7A) using Bx-Gal4 [17] was fully epistatic to the Su(H) gain of function phenotypes. This was in contrast to the simultaneous overexpression of UAS-rlsem with the UAS-Su(H) isoforms: in these experiments the Su(H) gain of function phenotypes prevailed (Figs. 6 and 7B). It has been described before that the overexpression of Su(H) in the developing sensory organs using sca-Gal4 causes a shaft to socket transformation [18], Fig. 3. Response of the Notch target gene vestigial. Overexpression of the UAS-Su(H) variants as indicated; the omb-expression domain is highlighted in blue in A–D and A″–D″ (A,A′ green fluorescent protein GFP; B–D and B″–D″, anti-Su(H) staining). Expression of the vestigial reporter vgBE-lacZ is shown in red (A–D″). UAS-GFP served as control. Note expansion of vgBE-lacZ expression along the dorso-ventral boundary (arrows) upon overexpression of Su(H) and Su(H)MAPK-ko, but not Su(H)MAPK-ac. Overgrowth of the ventral disc is marked by asterisks and is a consequence of the overexpression of Su(H) protein (B–D, B″–D″).
which we also observed upon overexpression of $\text{Su}(H)^{\text{MAPK-ko}}$ or $\text{Su}(H)^{\text{MAPK-ac}}$ (Fig. 8). Whereas $\text{sca}::\text{rlSEM}$ was similar to the control, $\text{sca}::\text{DERact}$ animals developed tufts of macrochaetae on the posterior thorax (Fig. 8). Interestingly, in combination with any of the $\text{Su}(H)$ variants, the double socket phenotype resulting from $\text{Su}(H)$ overexpression prevailed (Fig. 8). Finally, consequences of $\text{Su}(H)$ overexpression in the developing eye using $\text{gmr-Gal4}$ were addressed (Fig. 9). As the $\text{Gal4::UAS}$ system is temperature sensitive, phenotypes were strong at 29°C, revealing defects in the control as well [19]. At this temperature, $\text{Su}(H)$ overexpression caused overgrowth of the eye, irregular facets and necrosis. At 25°C the phenotypes were much weaker resembling the control. A combination with $\text{r}^{\text{SEM}}$...
enhanced the irregular facet and necrotic phenotype, whilst gmr::rSEM flies were very similar to the control (Fig. 9).

### 2. Experimental design, materials and methods

#### 2.1. Fly stocks, husbandry and analyses

Flies were obtained from the Bloomington stock collection if not noted otherwise. Fly husbandry was according to standard protocols at 29 °C, 25 °C or 18 °C as noted. y1 w1118, UAS-lacZ and UAS-GFP served as control. For information on fly stocks we refer to [http://flybase.bio.indiana.edu](http://flybase.bio.indiana.edu). Adult wings of female flies were dehydrated in ethanol and mounted in Euparal (Roth, Karlsruhe, Germany) and

![Expression of cut in response to Su(H), DERact and rSEM overexpression. Overexpression clones were induced in wing imaginal discs. They are labelled by the presence of GFP (green in A''–I''). Ectopic Su(H) protein is labelled in blue (A–I, A''–I''), and cut expression is shown in red (A–I, A''–I''). Constructs indicated at the left were under UAS-control. Note induction of cut upon overexpression of Su(H) (arrow in A'), but repression of cut by simultaneous overexpression of rSEM (C') labelled with blunt arrows. Likewise repression was seen in the combination with Su(H)MAPK-ac (D') but not or weakly in combination with Su(H)MAPK-ko (E'). DERact overexpression clones were frequently distorted and induced cut expression along the boundary to the non-overexpressing cells (arrowheads in F–I').](image)
dried over night. Pictures of wings or adult flies were taken on a Zeiss Axiophot or a Wild 5M stereomicroscope, respectively, using an ES120 camera (Optronics, Goleta CA, USA) and Pixera Viewfinder software, version 2.0.

Generation of UAS-Su(H)\(^{MAPK-ko}\), UAS-Su(H)\(^{MAPK-ac}\) and UAS-rlSEM was described earlier \cite{3,20}. UAS-rlSEM was provided by Martín-Blanco \cite{15} and UAS-DER\(^{act}\) by Freeman \cite{14}. LacZ-reporter gene constructs vg\(^BE\)-lacZ and E(spl)m8-lacZ were kindly provided by Bray and Schweisguth \cite{11,13}. Tissue-specific expression of respective transgenes was induced with the Gal4:: UAS-system \cite{6} using omb-Gal4 \cite{7}, Bx-Gal4 \cite{17}, sca-Gal4 \cite{21} and gmr-Gal4 \cite{19}. Overexpression clones were induced by the flip-out technique \cite{16} with the following fly lines: \(y\ w\ \text{flp}\(^{12}\);\) UAS-Su(H) or UAS-Su(H)\(^{MAPK-ko}\) mutants, \(y\ w\ \text{flp}\(^{12}\); UAS-rlSEM and \(y\ w\ \text{flp}\(^{12}\); UAS-rlSEM UAS-Su(H); UAS-DER\(^{act}\) and \(y\ w\ \text{flp}\(^{12}\); UAS-DER\(^{act}\) UAS-Su(H) or UAS-Su(H) mutants and \(y\ w\ \text{Act > CD2 > Gal4},\) UAS-GFP-nls (kindly provided by K. Basler).

Fig. 6. Overexpression consequences of Su(H), DER\(^{act}\) and rlSEM during thorax development. Co-overexpression of UAS-Su(H) variants together with UAS-lacZ (control), UAS-rlSEM or UAS-DER\(^{act}\) was driven in the developing thorax using Bx-Gal4 at 18 °C. Arrows point to examples of shaft to socket transformations that affected the majority of macrochaetae when UAS-Su(H) or UAS-Su(H)\(^{MAPK-ko}\) were overexpressed, but were rarely observed upon UAS-Su(H)\(^{MAPK-ac}\) ectopic expression. Simultaneous overexpression of UAS-rlSEM had little influence on each of these specific phenotypes. In contrast UAS-DER\(^{act}\) phenotypes were epistatic to the overexpression of any the respective Su(H) constructs, i.e. all the resultant flies resembled those of the single DER\(^{act}\) overexpression. Typical representatives are shown in each case.
Fig. 7. Overexpression consequences of Su(H), DERact and rlSEM during wing development. Co-overexpression of UAS-Su(H) variants together with UAS-lacZ (control), UAS-DERact (at 18 °C) (A) or UAS-rlSEM (at 25 °C) (B) was driven in the developing wing using Bx-Gal4. (A) At 18 °C, Su(H)MAPK-ko repressed vein formation (arrow) which was not observed for either Su(H) or Su(H)MAPK-ac. Induction of UAS-DERact resulted in very small wings mainly consisting of vein material, which was independent of Su(H) overexpression. As a consequence, the wings resulting from the combined overexpression were indistinguishable from those of the single DERact overexpression. (B) At 25 °C, overexpression of either Su(H) or Su(H)MAPK-ko but not Su(H)MAPK-ac induced tissue overgrowth typified by wing blisters (asterisks). Induction of UAS-rlSEM caused a network of veins (double arrowheads) which was repressed by the presence of ectopic Su(H) or Su(H)MAPK-ko but not by Su(H)MAPK-ac. At the same time Su(H) and Su(H)MAPK-ko gain of function phenotypes prevailed. Typical representatives are shown in each case.
2.2. Immunohistochemistry

Imaginal discs were stained according to standard protocols using mouse monoclonal antibodies directed against Cut, Wingless or beta-Galactosidase (developed by G.M. Rubin, S.M. Cohen, and J.R. Sanes respectively, and obtained from DSHB or using a polyclonal antiserum directed against Su(H)) [22]. Secondary antibodies coupled to FITC, Cy3 or Cy5 (1:200) were obtained from Jackson ImmunoResearch Laboratories (Dianova, Hamburg, Germany). Samples were mounted in Vectashield (Vector Lab) and examined on a Zeiss Axioskop coupled to a BioRad MRC1024 confocal microscope using LaserSharp 2000TM software (Carl Zeiss, Jena, Germany).
Acknowledgements

We are indebted to K. Basler, S. Bray, M. Freeman E. Martín-Blanco and F. Schweisguth for fly lines. Some antisera used in this work were obtained from the Developmental Studies Hybridoma Bank (DSHB) developed under the auspices of the NICHD and maintained by the University of Iowa, Dept. of Biology, Iowa City, Iowa 52242. The work was supported by the University of Hohenheim.

Fig. 9. Overexpression consequences of Su(H), DER\textsuperscript{act} and r\textsuperscript{SEM} in the developing eye. Co-overexpression of UAS-Su(H) variants together with UAS-lacZ (control) or UAS-rl\textsuperscript{SEM} was driven in the developing eye using gmr-X-Gal4. At 29 °C, gmr::lacZ flies have smaller eyes with irregular facets giving the eye a rough appearance. In contrast, overexpression of Su(H) variants at this temperature causes enlarged eyes that appear slightly bulgy. Both Su(H) and Su(H)MAPK-ko induced irregularities in the arrangement of the facets and necrosis (arrowhead), in contrast to Su(H)MAPK-ac. At 25 °C the phenotypes are much milder, and eyes appear like wild type (Su(H)MAPK-ac) or slightly rough (Su(H) and Su(H)MAPK-ko). A similar rough eye phenotype was observed upon induction of rl\textsuperscript{SEM} at 25 °C. The combined overexpression of Su(H) and rl\textsuperscript{SEM} gave a mixed phenotype, i.e. eyes were smaller, rough and necrotic (arrowhead). Similar necrotic patches (arrowhead) and size decrease were also observed in the eyes of gmr:: Su(H)MAPK-ko + rl\textsuperscript{SEM} animals, which in addition had a glossy appearance. In contrast, the eyes of the gmr:: Su (H)MAPK-ac + rl\textsuperscript{SEM} animals looked similar to gmr:: rl\textsuperscript{SEM}. Typical representatives are shown in each case.
Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2015.11.004.

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