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Early Expression of D-Pax2 in Drosophila Sense Organs is Controlled by a 700 Base Pair Cis-Regulatory Region

Conor Kirby OMS-I
University of Maine at Farmington; Marian University - Indianapolis

Joshua Kavaler
Colby College

Early Expression of D-Pax2 in *Drosophila* Sense Organs is Controlled by a 700 Base Pair Cis-Regulatory Region

Conor Kirby[1] and Joshua Kavaler[2]
[1]University of Maine at Farmington, Department of Natural Sciences, Farmington, Maine
[2]Colby College, Department of Biology, Waterville, Maine

Introduction

Bristles are mechanosensory organs that are an integral part of the *Drosophila* peripheral nervous system. They consist of four cells (shaft, socket, sheath, and neuron) that arise from a sensory organ precursor (SOP). D-Pax2 is a transcription factor necessary for the proper development of external sensory organs (Kavaler, et al. 1999). A 3.1 KB region upstream of D-Pax2 drives expression in a complete D-Pax2 pattern in both the early and late stages of bristle development. This region can be divided into a 2.2 KB region which drives early D-Pax2 expression and a 1 KB region that drives late D-Pax2 expression (Johnson, et al. 2011). The main objective of this research was to determine the minimal enhancer, the smallest DNA element sufficient to drive early or late expression of D-Pax2. We have cloned truncated fragments of both the 2.2 KB early and 1 KB late enhancers into a GFP reporter plasmid and used the plasmids to generate transgenic flies. These transgenic flies were dissected at early and late bristle stages and the efficacy of each fragment in driving gene expression was determined using expression of GFP in bristle cells as a readout.

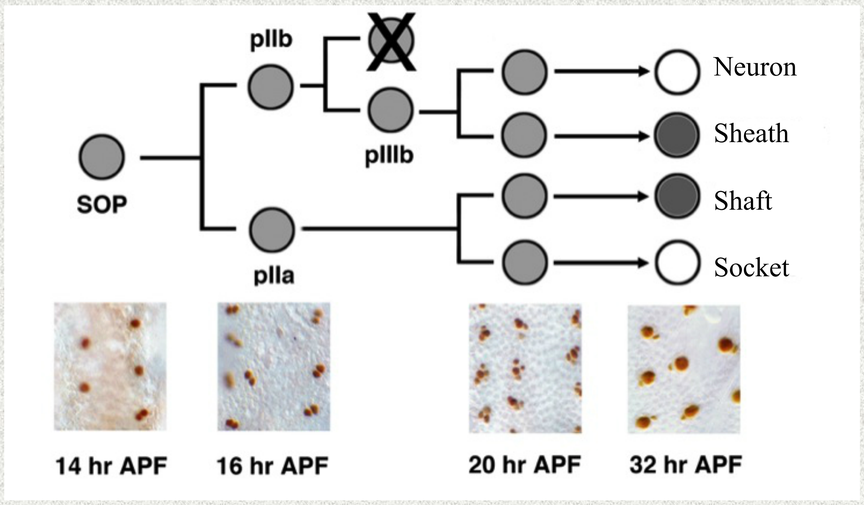

Figure 1: The development and differentiation of bristle cells on the *Drosophila* notum.

D-Pax2 protein expression is shown in all grey cells. Light grey indicates early expression during the mitotic phase, while dark grey indicates late expression during the differentiative phase. Late expression is restricted to the sheath and shaft cells. Images below diagram are pupal nota at the designated stages stained with an anti-D-Pax2 antibody (APF – after puparium formation).

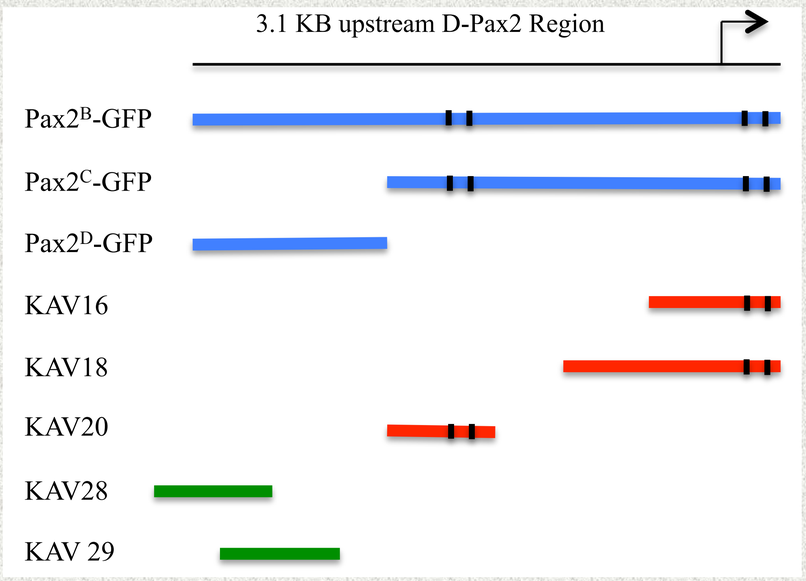

Figure 2: Map of GFP Reporter Constructs.

Colored lines represent enhancer regions cloned into a GFP reporter plasmid. Original constructs (Johnson et al., 2011) are in blue, and represent (in order): Full enhancer, early enhancer, and late enhancer. Early enhancer truncated constructs are shown in red, and late enhancer truncated constructs are shown in green. Proneural binding sites are shown as black stripes.

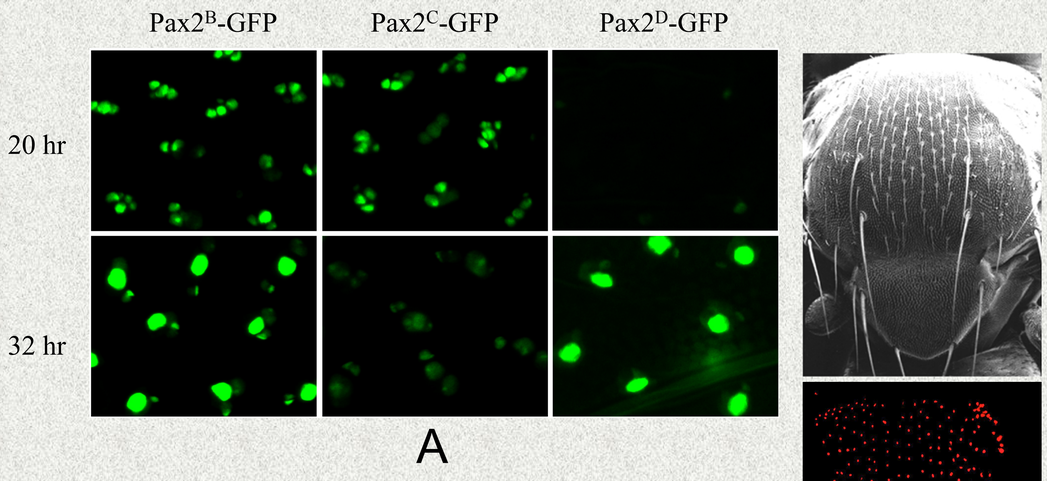

Figure 3: D-Pax2 expression is controlled by separate enhancer elements for early and late expression.

Expression of GFP in pupal nota from flies bearing the designated reporter constructs are shown above. Pax2^{B}-GFP controls all D-Pax2 expression, Pax2^{C}-GFP controls early expression, and Pax2^{D}-GFP controls late expression (A). An adult notum with bristles is pictured above corresponding to an anti-D-Pax2 staining that shows nuclei of bristle cells which will give rise to bristle development (B).

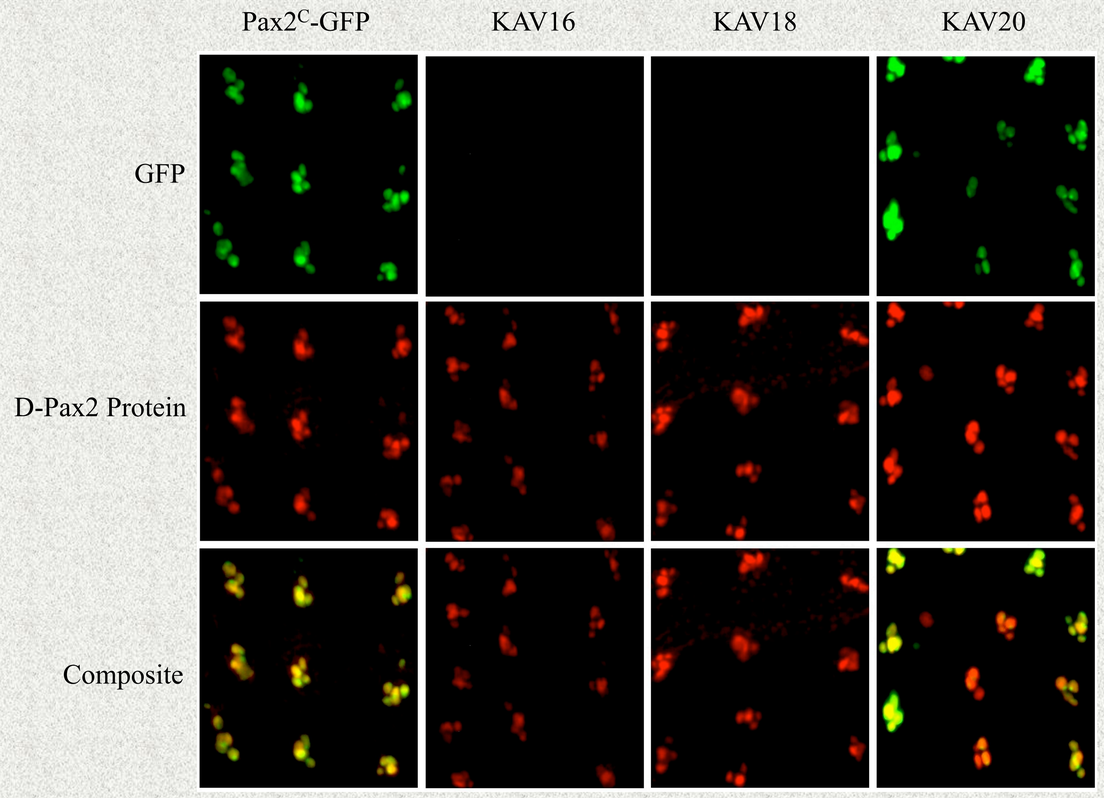

Figure 4: Early expression is controlled by a 700 base pair region.

Pupal nota bearing the designated reporter construct 20 hours APF. GFP expression is greatly reduced in KAV16 and KAV18. KAV20 shows similar GFP expression to Pax2^{C}-GFP. D-Pax2 protein (red) was detected with an anti-D-Pax2 antibody.

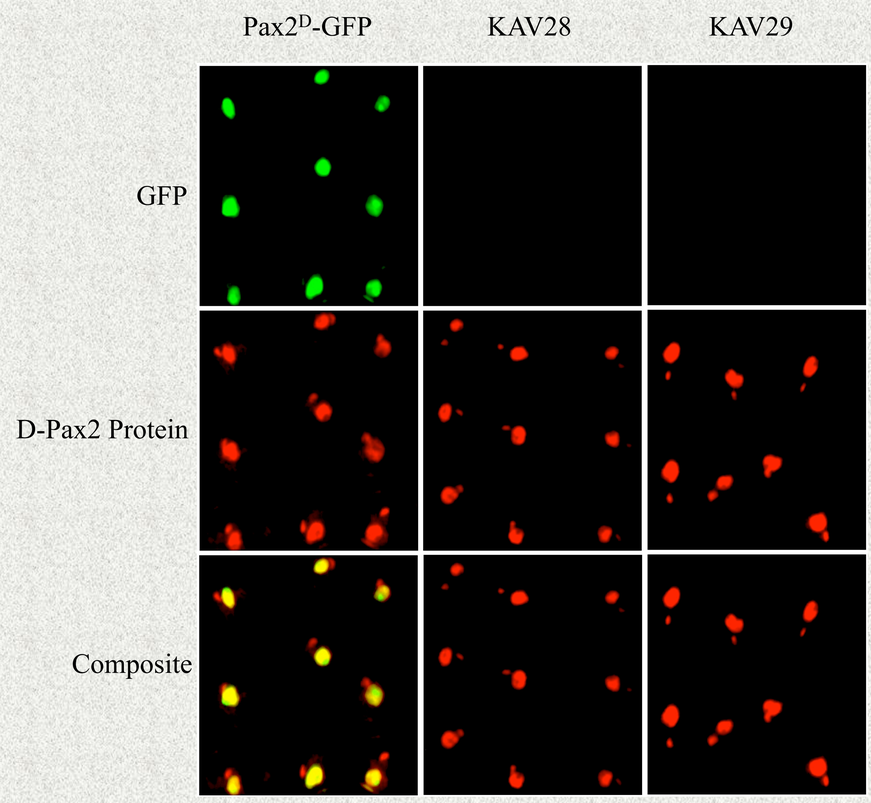

Figure 5: Late expression is greatly reduced when using KAV28 and KAV29 reporters.

Pupal nota bearing the designated reporter construct 32 hours APF. Lack of GFP expression in KAV28 and KAV29 compared to Pax2^{D}-GFP indicates that the reporter constructs do not have the full regions required for adequate expression.

Discussion

Loss of the more distal regions of the 2.2 KB early enhancer (KAV16, KAV18) abolishes expression. In contrast, loss of the region proximal to the D-Pax2 transcription start site has little effect on expression (KAV20). These results suggest that a small 700 base pair fragment including the two distal proneural binding sites is sufficient to drive early D-Pax2 expression. The two proximal proneural binding sites seem unnecessary for D-Pax2 expression.

Although the 700 base pair minimal enhancer and the 2.2 KB enhancer function similarly, expression levels between clusters varied noticeably for the minimal enhancer. A possible explanation is that some of the sequences removed from the 2.2 KB enhancer play a role in stabilizing consistent levels of expression.

We were unable to identify a minimal enhancer for late D-Pax2 expression by truncating either side of the 1 KB late enhancer (KAV28, KAV29). This indicates that late expression may require both distal and proximal regions of the enhancer. However, KAV28 and KAV29 both lack some of the proximal region of the late enhancer, so expression may be controlled in part by that region.

Works Cited

Johnson, SA, Harmon, KJ, Smiley, SG, Still, FM, and Kavaler, J. "Discrete Regulatory Regions Control Early and Late Expression of D-Pax2 During External Sensory Organ Development." *Developmental Dynamics* 240 (2011): 1769-1778.

Kavaler, J, Fu, W, Duan, H, Noll, M, and Posakony, JW. "An essential role for the Drosophila Pax2 homolog in the differentiation of adult sensory organs." *Development* 126 (1999): 2261-2272.

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