

Chapter 5

A Dose Dependent Screen for

Modifiers of Kek5

ABSTRACT

Modifier screens in *Drosophila* have proven to be a powerful tool for uncovering gene interaction and elucidating molecular pathways. Misexpression of Kek5 causes a number of adult phenotypes, of which the most overt are a rough eye and the scutellar bristle duplication. A prior modifier screen carried out by T. Evans using the rough eye phenotype proved unsuccessful (Evans and Duffy, 2006). As described in chapter three, alterations in Kek5 activity led to modifications in the scutellar bristle duplication phenotype, confirming that this phenotype is sensitive to the levels of Kek5. Therefore to identify potential interactors of Kek5, a dose dependent, genome wide deficiency screen for modifiers of the Kek5 bristle phenotype was undertaken. From this screen, four potential modifiers caused a modification of the bristle duplication phenotype, however, the effects could not be mapped to specific loci.

INTRODUCTION

The ability to perform genetic screens in *Drosophila* has helped uncover components of various biological pathways. Modifier screens, in particular, have helped elucidate signal transduction pathways (Johnston, 2002). In a modifier screen, one is looking for an enhancement or suppression of a particular phenotype associated with the gene of interest in a sensitized genetic background. To identify molecules that interact with Kek5, I undertook a dose-dependent modifier screen using deficiencies to halve the dose of a particular genomic region and assess its effect on a Kek5 dependent phenotype. Although a complete loss of *kek5* does not result in overt phenotypes, misexpression of Kek5 does result in phenotypes with high penetrance.

Previously, a rough eye phenotype due to misexpression of Kek5 in the *Drosophila* eye using *GMR>kek5* was used in a modifier screen (Evans and Duffy, 2006). This screen uncovered *wing blister* (*wb*), a member of the integrin pathway, as a potential enhancer of the rough eye phenotype of *GMR>kek5* (Martin et al., 1999). In the current study, I used the Kek5 gain of function scutellar phenotype (ectopic bristles), which is sensitive to Kek5 activity and can be easily quantified, as the basis for a modifier screen. Coupled with the knowledge that BMP signaling has been shown to affect bristle patterning and that Kek5 modulates BMP signaling, the hope was that using the scutellar phenotype might provide insight to Kek5's role in BMP signaling (Tomoyasu et al., 1998; Wharton et al., 1999). The logic was that halving the dose of a defined chromosomal region that interacted with Kek5 would result in alteration in the number of bristles based on the type of interaction (Figure 5.1). The screen uncovered two modifiers that enhanced the Kek5 bristle phenotype and two that suppressed it. However these effects were unable to be mapped to single genes.

RESULTS

In the current study, the scutellar bristle duplication phenotype displayed by *ptc>kek5*, a phenotype that is dose sensitive, was utilized in a deficiency based modifier screen. At 25°C *ptc>kek5* has an average of nine scutellar bristles as opposed to four present in wild type flies (or up to six in *ptcGAL4* alone). This number increases to an average of fifteen when the temperature is raised to 28°C. The screen was performed at 25°C to ensure suppression as well as enhancement of the bristle duplication phenotype could be detected.

The screen was performed in a *ptc>kek5* background which results in misexpression of Kek5 in the anterior-posterior boundary of the imaginal discs and drives expression in the notum region of the wing disc that gives rise to the adult scutellum (Figure 5.1). A strain in which the *ptcGAL4* insertion was recombined with the *UAS-kek5•GFP* insertion was mated with the chromosomal deficiency stocks at 25°C. FI females containing the *ptc>kek5* and a single copy of deficiency were scored for the number of scutellar bristles and screened for enhancement or suppression of the bristle duplication phenotype (Figure 5.1). A total of 311 deficiencies from the *Drosophila* Stock Center were selected to attain maximum chromosomal coverage and covered the I, II and III chromosomes (Table 5.1a and Table 5.1b).

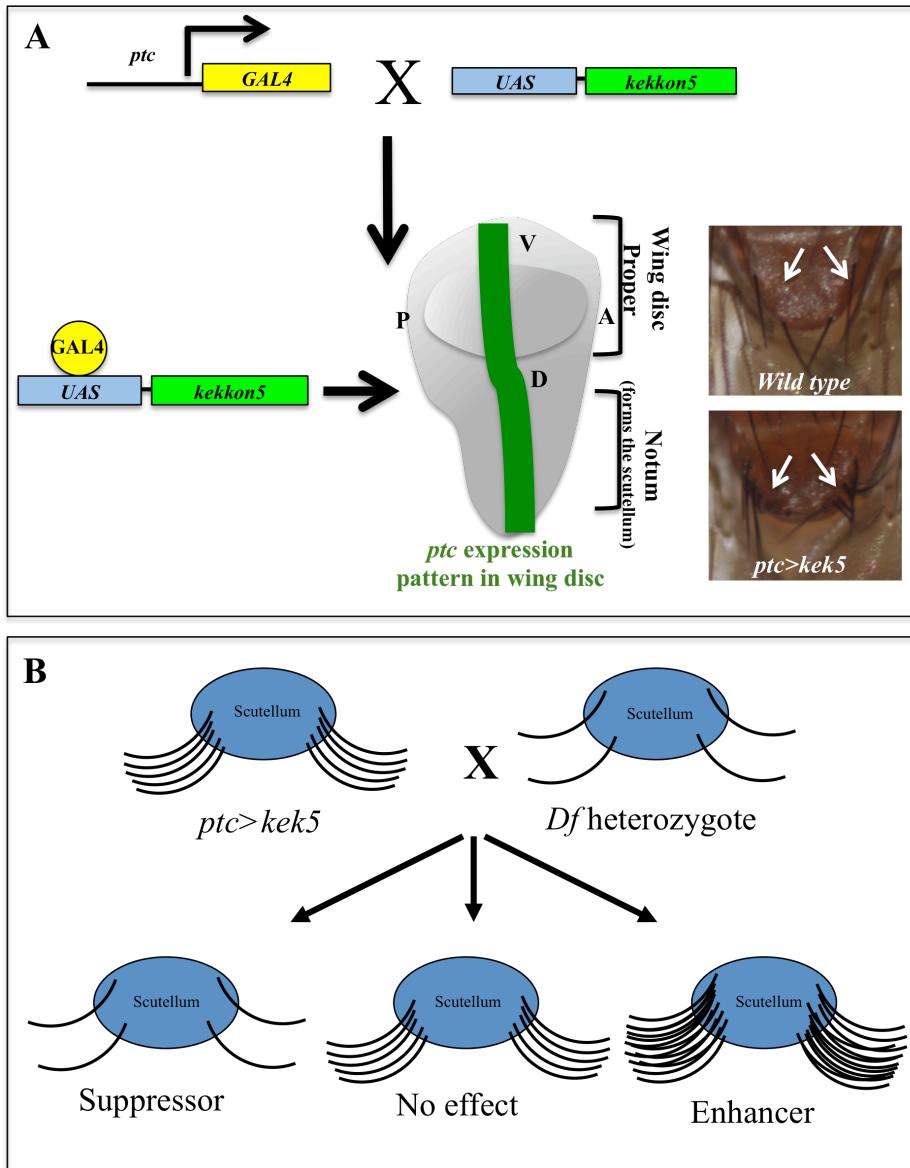


Figure 5.1: Schematic diagram of the deficiency screen. A) Kek5.GFP was expressed in the *ptc* expression domain using the UAS/GAL4 system resulting in a duplication of the scutellar bristles. Ptc expression domain lies just at the anterior side of A/P boundary. A-Anterior; P-Posterior; V-Ventral; D-Dorsal. B) For the screen *ptc>kek5* recombinant was crossed to the various deficiencies listed in Table 6.1a and 6.1b to look for suppressors or enhancers of the scutellar bristle duplication phenotype.

During the screen, I observed lethality with five deficiencies *Df(2L)Exel6011*, *Df(2R)Exel6072*, *Df(2L)Exel6027*, *Df(3R)Exel6159* and *Df(2R)Exel7171* (Table 5.1a). However, when these deficiencies were crossed with *ptcGAL4* alone they also exhibited lethality indicating

the interactions were between the deficiencies and *ptcGAL4* and were not due to Kek5 misexpression.

The results of the screen are graphed in a frequency chart (Figure 5.2) Deficiencies at the extreme ends (total of 4) of the graph showed the strongest effects and were focus of my study. These outliers included two suppressors (suppressing the number of scutellar bristles from 9 to ~3) in the chromosomal region 66A17-66B5 and 89B14-89B19 and two enhancers in regions 21B3-21B7 and 85A5-85D1 (enhancing the number of scutellar bristles from 9 to 14 and 13.57, respectively). The regions uncovered by these deficiencies did not include any obvious candidates for interactors of Kek5, such as components of BMP signaling pathway or the cell adhesion molecules of the integrin and the Cadherin–Catenin complex (Table C1).

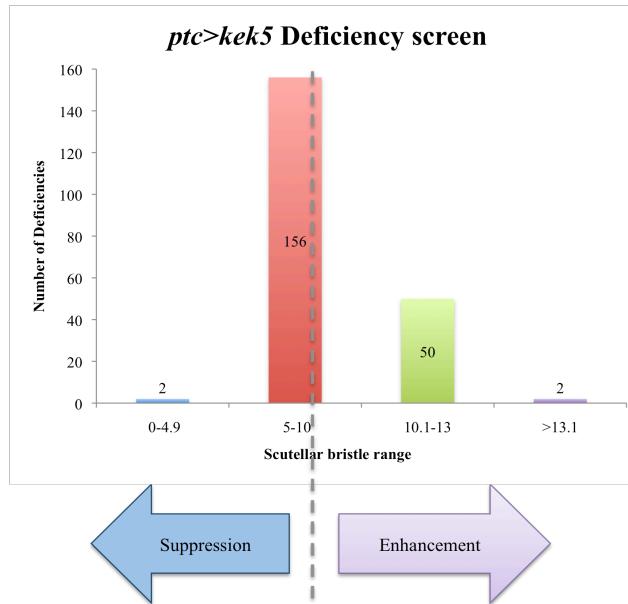


Figure 5.2: Frequency distribution chart showing the results of the *ptc>kek5* deficiency screen. The number in each bar indicates the number of deficiencies that fell in that range. Grey dotted line indicates the number of bristles in *ptc>kek5* at 25°C.

To ensure that the drastic alteration seen in the number of scutellar bristles was due to the genes deleted in the region deleted in a particular deficiency stock and not due to additional mutations in the genetic background of that particular stock, overlapping deficiencies covering the region were tested for all 4 stocks (Figure 5.3 and Figure 5.4). None of the overlapping deficiencies tested were able to replicate the results obtained for the corresponding original deficiency. One possible explanation is that there were additional mutations in the genetic

background of the four initial deficiency stocks that were responsible for the associated suppression or enhancement. The complete table with the analysis of overlapping deficiencies and alleles of genes in the 4 respective regions that were tested can be found in Table C2.

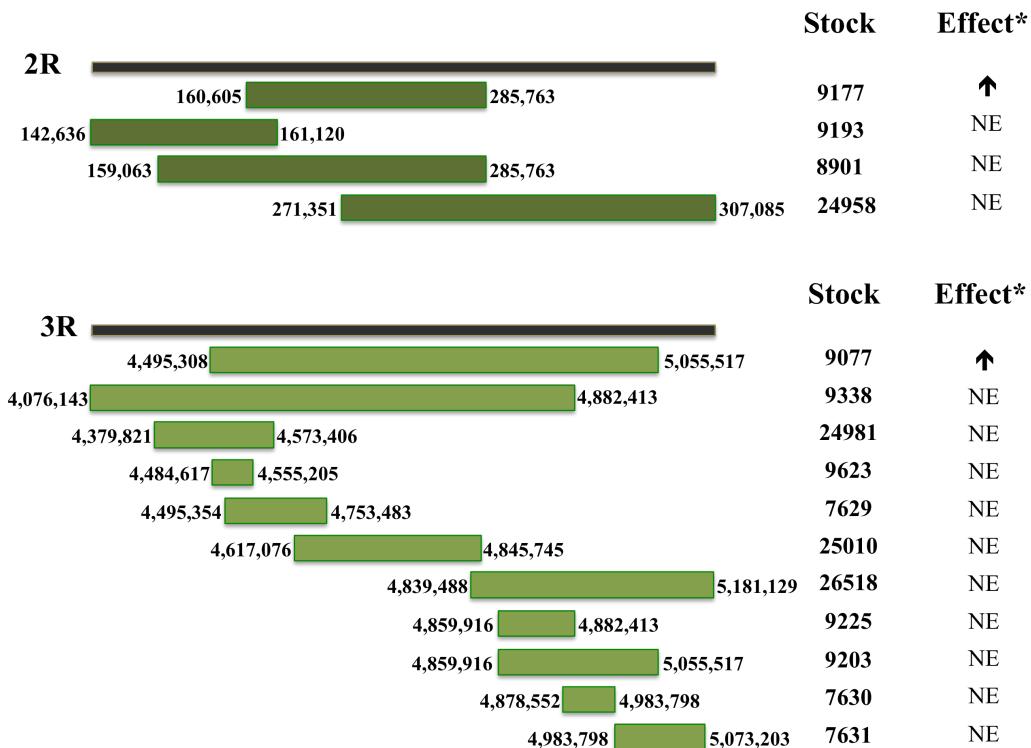


Figure 5.3: Overlapping deficiencies tested for stocks that enhanced the *ptc>kek5* bristle phenotype. Bars indicate the chromosomal region deleted in the corresponding stock. Dark green bars indicate deficiencies tested for 9177 while light green bars indicate deficiencies tested for 9077. Numbers at either end of the green bars indicate the chromosomal break points. * Effect on Kek5 bristle duplication.

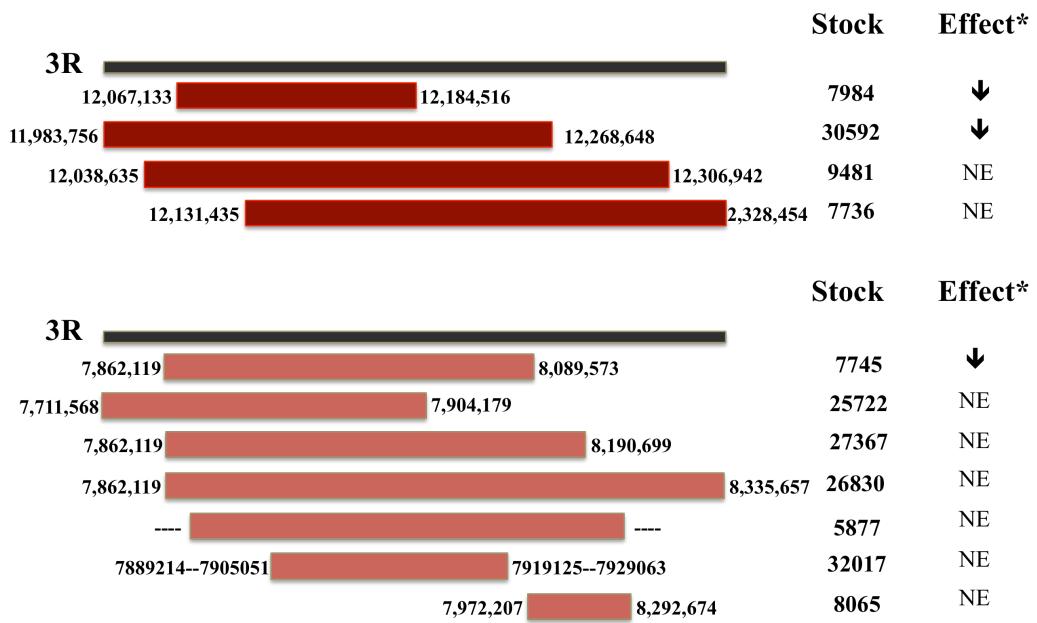


Figure 5.4: Overlapping deficiencies tested for stocks that suppressed the *ptc>kek5* bristle phenotype. Bars indicate the chromosomal region deleted in the corresponding stock. Red bars indicate deficiencies tested for 7984 and orange bars indicate deficiencies tested for 7745. Numbers at either end of the bars indicate the chromosomal break points. * Effect on Kek5 bristle duplication.

Table 5.1b: Deficiency screen looking for modifiers of scutellar bristle duplication phenotype displayed by *ptc>kek5* – Part II.
 Interesting deficiencies are highlighted (bold).

X Chromosome

BS #	Deletion Name	Deletion Cytology	Average # of bristles
1 Ptc>K5/+	CONTROL	-	8.37
2 Ptc>K5/+	CONTROL	-	10
7699	Df(1)Exel6221	1B4--1B8	5.95
9052	Df(1)ED6396	1B5--1B8	6.5
9053	Df(1)ED6443	1B14--1E1	8
7702	Df(1)Exel6225	1D4--1E3	9.21
7703	Df(1)Exel6226	1E3--1F3	8.75
7704	Df(1)Exel6227	1F3--2B1	9
7769	Df(1)Exel8196	2B1--2B5	11.33
9054	Df(1)ED6574	2E1--3A2	9.24
7705	Df(1)Exel6230	3A2--3A4	5
8031	Df(1)ED411	3A3--3A8	9.22
9348	Df(1)ED6584	3A8--3B1	9
8948	Df(1)ED6630	3B1--3C5	7.28
7707	Df(1)Exel6233	3D2--3D4	9.5
9169	Df(1)ED6712	3D3--3F1	15
24145	Df(1)ED6716	3F2--4B3	7.62
9055	Df(1)ED6720	4B3--4C7	6.25
8956	Df(1)ED6727	4B6--4D5	8.55
7753	Df(1)Exel6290	4F7--4F10	7.93
7708	Df(1)Exel6234	4F10--5A2	9.22
7709	Df(1)Exel6235	5A2--5A6	10.14
8949	Df(1)ED6802	5A12--5D1	8
8947	Df(1)ED6829	5C7--5F3	7.1
7713	Df(1)Exel6239	5F2--6B2	10.5
7714	Df(1)Exel6240	6B2--6C4	7.58
23670	Df(1)BSC285	6C11--6D3	10.38
9625	Df(1)ED6878	6C12--6D8	9.75
8955	Df(1)ED6906	7A3--7B2	9.06
7715	Df(1)Exel6241	8A2--8B2	7.5
8033	Df(2L)Exel8033	35B1--35B8	7.19
7770	Df(1)Exel9049	8D2--8D3	9.82

BS #	Deletion Name	Deletion Cytology	Average # of bristles
9153	Df(1)ED7005	9B1--9D3	7.91
9057	Df(1)ED7010	9D3--9D4	8.35
23672	Df(1)BSC287	10A10--10B11	10.8
9154	Df(1)ED7067	10B8--10C10	8.95
7716	Df(1)Exel6242	10D1--10D7	11.46
9171	Df(1)ED7147	10D6--11A1	7.4
9217	Df(1)ED7161	11A1--11B14	7.33
8898	Df(1)ED7170	11B15--11E8	7.2
7718	Df(1)Exel6245	11E11--11F4	7.25
8952	Df(1)ED7217	12A9--12C6	9.38
24146	Df(1)ED7225	12C4--12E8	9.75
9352	Df(1)ED7229	12E5--12F2	7.38
9218	Df(1)ED7261	12F2--12F5	10
24336	Df(1)BSC310	12F5--13A10	9.8
8035	Df(1)ED7294	13B1--13C3	10.45
9219	Df(1)ED7331	13C3--13F1	10.55
7720	Df(1)Exel6251	13F1--13F17	10
9905	Df(1)ED7364	14A8--14C6	5.17
8954	Df(1)ED7374	15A1--15E3	8.7
24429	Df(1)BSC405	16D5--16F6	9.2
24376	Df(1)BSC352	16F7--17A8	6.6
8036	Df(1)ED447	17C1--17F1	5.85
7754	Df(1)Exel6291	18A2--18A3	9.69
8951	Df(1)ED7441	18A3--18C2	7.05
23171	Df(1)BSC275	18C8--18D3	11.63
7721	Df(1)Exel6253	18D13--18F2	8.43
9351	Df(1)ED7635	19A2--19C1	9.3
7722	Df(1)Exel6254	19C4--19D1	8.3
9172	Df(1)ED7664	19F1--19F6	10.42
7723	Df(1)Exel6255	20A1--20C1	8
9156	Df(1)ED12432	20C3--20D2	9.39
9346	Df(1)ED14021	20C3--20F1	7.45

8104	Df(3R)ED5780	89E11;90C1	10.85
9208	Df(3R)ED5815	90F4;91B8	8.58
6962	Df(3R)ED2	91A5;91F1	9.87
8964	Df(3R)ED6025	92A11;92E2	10.94
9487	Df(3R)ED10845	93B9;93D4	12.17
8962	Df(3R)ED6076	93E10;94A1	12.35
8923	Df(3R)ED6085	93F14;94B5	10.4
8684	Df(3R)ED6096	94B5;94E7	6.74
8963	Df(3R)ED6103	94D3;94E9	6.6
7990	Df(3R)Exel9012	94E9;94E13	7.84
7991	Df(3R)Exel9013	95B1;95B5	9.33
7992	Df(3R)Exel9014	95B1;95D1	9.71
9211	Df(3R)ED6220	96A7;96C3	9.9
7994	Df(3R)Exel9056	96C4;96C5	7.95
8105	Df(3R)ED6232	96F10;97D2	9.87
9478	Df(3R)ED6235	97B9;97D12	8.17
9210	Df(3R)ED6255	97D2;97F1	8.79
8960	Df(3R)ED6265	97E2;98A7	8.33
8961	Df(3R)ED6310	98F12;99B2	10.35
8925	Df(3R)ED6316	99A5;99C1	11
7997	Df(3R)Exel7378	99F8;100A5	8.33
7919	Df(3R)Exel7379	100B2;100B3	6.52

DISCUSSION

Altering the levels of Kek5 has been shown to have a multitude of effects such as defects in crossvein patterning (BMP signaling), scutellar bristle duplication and cellular defects (Arm upregulation, epithelial cell extrusion and large cell) (Evans et al., 2009; this work). In an attempt to identify molecules that interacted with Kek5, a genome-wide deficiency screen was performed to look for modifiers of the *ptc>kek5* bristle duplication phenotype. The reasons for selecting this phenotype were its overt and quantifiable nature and sensitivity to variation in levels of Kek5. By varying the amount of GAL4 produced (by changing the temperature), the strength of the phenotype could be varied; 9 bristles at 25°C increases to 15 bristles at 28°C in *ptc>kek5* flies. Thus, we were hopeful to uncover modifiers of Kek5 activity through this screen. However, while the screen uncovered two suppressing and two enhancing strains, these effects could not be mapped to single loci. One possible explanation is that the observed modifications were due to something in the genetic background of the respective stocks, specifically on the deficiency chromosome, but distinct from the region uncovered by the deficiency itself.

MATERIALS AND METHODS

Drosophila genetics

All the screen crosses were performed at 25°C. The screen was performed by mating *ptc>kek5* recombinant virgins with males from the deficiency stocks and *ptc>kek5/Df* F1 progeny were examined for the number of scutellar bristles. Deficiency stocks used in the screen are listed in Table 5.1a and Table 5.1b.

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