From [redacted] **Tue May 14 13:20:16 1996**
From: Rachel Drysdale (Genetics) [redacted]
To: [redacted]
Cc: [redacted]
Subject: region 55 manuscript found
Date: Tue, 14 May 96 13:20:06 BST

Hi Rick,

Sarah Bray has just found a copy of your manuscript, so you don't need to keep looking for it.

I will curate it as a personal communication from you to FlyBase, if that is OK.

with best wishes,

Rachel

Rachel Drysdale.

FlyBase (Cambridge),
Department of Genetics,
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From [redacted] Tue May 14 14:27:29 1996
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Date: Tue, 14 May 1996 22:50:03 +0930
To: Rachel Drysdale (Genetics) [redacted]
From: [redacted] (Rick Tearle)
Subject: Re: region 55 manuscript found
Content-Length: 209

Dear Rachel,

Thank you for your email re the region 55 paper. Curate away!

Rick

Rick Tearle
Xenotransplantation Lab
Bresagen Ltd
[redacted]
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Fx: [redacted]

The cytological region uncovered by Df(2R)PC4 (55A to 55C) contains three genes of developmental interest. The maternal effect locus staufen (stau) is required for abdominal segmentation and pole cell formation (Schüpbach and Wieschaus 1986). In the absence of maternally supplied gene product, most abdominal segments and the pole cells fail to form . The anteriorly located head skeleton is also reduced. The zygotic locus three rows (thr) is required for completion of post blastoderm mitoses. Cells in thr⁻ embryos progress through to metaphase of the 14th cell division quite successsfully but fail to undergo the following anaphase (C. Lehner pers. comm.). The third gene, Polycomblike (Pcl), is required for the correct expression of the homeotic genes in the bithorax and Antennapedia gene complexes (Duncan 1982; Jürgens 1986). In the absence of zygotically expressed gene product the abdominal segments adopt identities of segments more posterior to them. Pcl is also haplo insufficient, with males carrying only one functional copy of the gene occasionally developing sex combs on their second and third legs.

Several other embryonic lethal and maternal effect genes have also been mapped to this cytological region: grainy head (grh; called l(2)IM45 in Nüsslein Volhard, Wieschaus and Kluding 1984), early (eay), subito (sub), halted (hal) and maternal(2)late oogenesis-DF6 (mat(2)lto-DF6) (Schüpbach and Wieschaus 1989; T. Schüpbach pers. comm.). The relative abundance of genes in the region with a maternal or embryonic lethal phenotype, the need to recover further alleles at some of these loci and the occasional clustering of genes with similar function in D. melanogaster prompted us to undertake a detailed genetic analysis of the region. We therefore carried out an EMS mutational screen for visible, maternal effect and zygotic lethal loci by screening against the deficiency Df(2R)PC4 (55A;55C).

Only the zygotic lethal screen was successful, uncovering 19 loci in this cytological region. The maternal component of the screen was unsuccessful in that it failed to uncover futher alleles at two of the four previously known loci but it did provide us with two more maternal effect loci. All of the loci were mapped with respect to six deficiencies which overlap Df(2R)PC4. Surpisingly the number of alleles per locus differed markedly between different regions, with the bulk of the loci mapping to distal end of Df(2R)PC4 being represented by only one allele.

Fly Stocks: Most of the markers, cytological aberrations and balancer chromosomes are described in Lindsley and Grell (1968), Lindsley and Zimm (1984) or Steward and Nüsslein-Volhard (1986). Alleles of thr, Pcl, grh and stau were recovered in previous screens in this laboratory (summarised in Tearle and Nüsslein-Volhard 1989), or were provided by T. Schüpbach. Df(2R)Pcl-XM82 was isolated by G. Jurgens (unpublished). Df(2R)Pcl-7B and Df(2R)Pcl-7B were provided by I. Duncan. Df(2R)P29, Df(2R)P29, and Df(2R)P29 were provided by ?.

Fly Food: Flies were grown on standard medium as described in Steward and Nüsslein-Volhard (1986).

EMS Mutagenesis: A second chromosome marked with black purple cinnabar waxy^{waxlex} brown (b pr cn wx^{wxl} bw) was isogenised. When egg lays were tested, over 95% of embryos homozygous for this chromosome successfully completed embryonic development. About 1,000 homozygous b pr cn wx^{wxl} bw males were treated with ethyl methane sulphonate (EMS) essentially as described by Lewis and Bacher (1968). After 24hr exposure to EMS the males were mated to CyO,DTS100/Bl1f females in aliquots of 100 pairs per bottle. The flies were transferred to fresh bottles every 2 days for 8 days and then discarded. CyO,DTS100/b pr cn wx^{wxl} bw F_1 males and virgin females were collected and individually mated to 2-3 CyO/Df(2R)PC4 or CyO,bw/Df(2R)PC4,exu^{P1} bw males or females. The vials were incubated at 29° for several days to kill animals carrying the temperature sensitive CyO,DTS100 chromosome. Upon eclosion flies were transferred to egg laying blocks for analysis of female fertility. Where more than 25% of the eggs failed to hatch, the blocks were checked for the presence of Df(2R)PC4/b pr cn wx^{wxl} bw F_2 parents. If found, these were set up independently of their siblings to determine whether the female sterility was likely to be due to a mutation on the b pr cn wx^{wxl} bw chromosome. After testing for female sterility was completed, the F_2 vials were examined for the presence of mutant Df(2R)PC4/b pr cn wx^{wxl} bw (visible mutations) or the absence of this genotype altogether (zygotic lethal mutations). Where a visible, female sterile or zygotic lethal mutation was suspected stocks were set up with CyO/b pr cn wx^{wxl} bw flies.

Cytological mapping of mutations: Putative mutations were rechecked against CyO/Df(2R)PC4 and (along with extant mutations) crossed to the three deficiencies Df(2R)Pcl-XM82, Df(2R)Pcl-7B, and Df(2R)Pcl-11B. When this screen was undertaken, only these deficiencies were available All three map to the proximal end of Df(2R)PC4, so mutations which are viable over them and lethal over the Df(2R)PC4 bearing chromosome could map either to the distal end of this deficiency, or to other lethals carried by the chromosome. To separate such lethals into these two classes, they were crossed to Df(2R)Pcl-XM82 and resultant Df(2R)Pcl-XM82/l(2)x females then backcrossed to Df(2R)PC4. Crosses giving chromosomes bearing neither the deficiency nor the lethal were taken to indicate that the lethal lay outside Df(2R)PC4. The remainder were assumed to map within Df(2R)PC4, an assumption that is supported by the failure of these lethals to complement three recently generated deficiencies which map to the distal end of Df(2R)PC4): Df(2R)P29, Df(2R)P34, and Df(2R)P66.

Chromosome Cytology: 3-4 Males carrying deficiency bearing chromosomes over CyO were crossed to 5-6 Canton S females. Larvae were grown at 18° and yeasted well. Polytene chromosomes were obtained from salivary glands of wandering 3rd instar larvae and prepared by standard methods. Chromosomes were stained with lacto-aceto-orcein and examined with phase optics. Assignment of breakpoints was made using Lindsley and Grell (1968) and Lefevre (1976).

Determination of lethal phase and embryonic phenotype: For lethal mutations, the time of lethality was determined by crossing stocks to CyO/Df(2R)PC4 and counting the number of progeny that failed to hatch (CyO homozygotes die as first instar larvae). Where this did not differ significantly from 25% the mutation was considered embryonic lethal. For female sterile and embryonic lethal mutations, embryonic cuticle preparations were prepared essentially as described in Wieschaus and Nüsslein Volhard (1986).

Frequency of lethal mutations: To determine the average number of lethal hits per chromosome, CyO/b pr cn wx^{wxl} bw flies were taken from 100 F_2 vials selected at random and transferred to fresh vials. Their progeny were examined and the proportion containing homozygous (i.e. lethal free) b pr cn wx^{wxl} bw flies calculated. We assume that lethal hits per chromosome follow a Poisson distribution and can estimate the average number by using the expression $P(0)=e^{-m}$ where P(0) is the fraction without lethal hits and m is the average number of lethal hits per chromosome.

Isolation of lethal and female sterile mutations: 7784 flies carrying an EMS treated b pr cn wx^{wxt} bw bw chromosome were crossed to CyO/Df(2R)PC4 and tested for lethality and female sterility. 4659 lines produced sufficient progeny to be tested; of these 55 carried lethal mutations and 5 carried female sterile mutations that failed to complement the Df(2R)PC4 bearing chromosome. 41 of the lethal mutations mapped to the region uncovered by Df(2R)PC4 itself, the remainder mapped outside the deficiency and presumably represent extraneous lethals on the deficiency chromosome. These 44 true Df(2R)PC4 region mutations (41 lethals and 5 female steriles) were initially crossed to 3 other deficiencies which overlapped or were contained in Df(2R)PC4: Df(2R)Pcl-7b, Df(2R)Pcl 11b and Df(2R)Pcl-XM82. This allowed us to assign small groups of mutations to different chromosomal intervals as defined by the deficiencies. Mutants mapping to different intervals were then crossed inter se, and then to previously recovered mutations which were mapped to the same intervals. We were thus able to establish complementation groups across the region.

The 41 lethal mutations uncovered in this screen represent 19 complementation groups. The 5 female sterile mutations recovered represent 4 separate loci, only 2 of which were previously known, and bring the number of female sterile loci in the region to 7. The mutations map to the following intervals:

Region 1: This region is defined by the left breakpoint of Df(2R)PC4 and the right breakpoint of Df(2R)Pcl-7b. Loci mapping to this region fail to complement all four deficiencies. They are: Pcl, thr, l(2)PC4-A, l(2)PC4-B stau and cay. Alleles were recovered for all three of the pre-existing lethal loci, Pcl, thr and l(2)PC4-A. [The l(2)PC4-A locus was previously represented by the allele l(2)HD53 (T.Schüpbach pers. comm.)]. On average 3 alleles were recovered per lethal complementation group. Two alleles of the maternal locus, cay, were recovered, and none for stau.

Region 2: This region is defined by the right breakpoint of Df(2R)Pcl-11B and the right breakpoint of Df(2R)Pcl-7b. Loci mapping to this region fail to complement Df(2R)PC4, Df(2R)Pcl-XM82 and Df(2R)Pcl-7b. They are: grh, l(2)PC4-C and l(2)PC4-D. grh was previously described on the basis of one allele and 4 more alleles were recovered in this screen. Three of them are also embryonic lethal in trans to the original grh allele and show the same degenerating head skeleton. For this region 3 alleles on average were recovered per lethal complementation group

Region 3: This region is defined by the right breakpoint of Df(2R)Pcl-7B and the right breakpoint of Df(2R)Pcl-XM82. Loci mapping to this region fail to complement Df(2R)PC4 and Df(2R)Pcl-XM82. They include 9 previously undescribed loci - l(2)PC4-E to l(2)PC4-M and fs(2)PC4-N. Three female sterile loci mapping to this region have been previously described, hal, mat(2)lto-DF6 and sub (Schüpbach and Wieschaus 1989). One allele of sub was recovered in this screen. [The nature of the mutagenesis screen would not permit the recovercy of alleles for hal as mutant females fail to lay eggs (T. Schüpbach pers. comm.)]. For 6 of the 8 lethal loci only one allele was recovered and on average only 1.6 alleles were recovered per lethal complementation group.

Region 4: This region is defined by the right breakpoint of Df(2R)Pcl-XM82 and the right breakpoint of Df(2R)Pcl-PC4. Loci mapping to this region fail to complement Df(2R)PC4 alone. They include 5 previously undescribed loci - l(2)PC4-P to l(2)PC4-S and fs(2)PC4-T. These loci were further tested for complementation with 3 deficiencies that fail to complement Df(2R)Pcl-PC4 but do complement Df(2R)Pcl-XM82, Df(2R)Pcl-7B and Df(2R)Pcl-11B. They are Df(2R)P29, Df(2R)P34 and Df(2R)P66. Three loci, l(2)PC4-P, l(2)PC4-Q and l(2)PC4-S map to the region uncovered by all three deficiencies. The remaining lethal locus, l(2)PC4-R fails to complement Df(2R)P34 and Df(2R)P66 but does complement Df(2R)P29. The placement of of loci withi this region is uncertain because Df(2R)x lies within Df(2R)PC4. For 3 of these 4 lethal loci more than one allele was recovered in this screen but on average only 1.8 alleles were recovered per lethal complementation group.

Number of Lethal Hits per Chromosome: To establish the degree of saturation of the region achieved in this screen, we first estimated the relative number of lethal hits per mutagenised chromosome. 100 of the 4569 lines were taken at random and checked for homozygous b pr cn wx^{WXI} bw progeny. 23 lines produced homozygous progeny. Assuming that the number of lethal hits per chromosome follows a Poisson distribution, this represents 1.47 hits per chromosome. There are approximately 1700 loci on the second chromosome that can mutate to lethality (Anderson and Nüsslein Volhard) and we analysed 4569 chromosomes, so on average every lethal locus was hit 3.9 times.

Asymmetrical Distribution of Alleles Recovered: The distribution of alleles for the lethal loci shows a distinctly asymmetrical distribution. For the 7 loci in regions 1 and 2, 3.0 alleles were recovered per locus. For the 13 loci in regions 3 and 4 this drops to 1.6 and 1.8. All 8 lethal loci which are represented by only one allele map to regions 3 and 4 (out of 12 lethal loci in these regions).

DISCUSSION

The one of the main aims of this screen was to saturate the region uncovered by Df(2R)PC4 for lethal and maternal loci. We have probably identified most, if not all, of the lethal loci mapping to this deficiency. We recovered 41 lethal mutations which map to 19 loci, an average of 2.2 alleles per locus. This is less than that expected when compared to the average number of hits per locus of 3.9 for the whole chromosome. The reason for this discrepancy is not obvious. The frequency of alleles recovered is further confounded by the distinct bias in alleles per locus for different regions. The seven loci in regions 1 and 2 are represented here on average by 3 alleles, region 3 by 1.6 alleles and region 4 by 1.8 alleles. This bias is even more pronounced when the number of loci represented by only one locus is considered. Seven of 8 such loci are clustered in region 3, with the remaining locus found in region 4. We can see no reason inherent in the design of this mutational screen which would explain these results. We are left with the possibility that Region 3 contains an abnormally high number of loci which are relatively resistant to EMS mutagenesis. Unfortunately EMS mutagenesis for lethal loci in this region is unlikely to be repeated.

Of the 19 lethal loci, 5 are embryonic lethal. This is consistent with previous data for virtually the whole genome which suggests that about 25% of all lethal loci are embryonic lethal (Nüsslein-Volhard, Wieschaus and Kluding 1984; Jürgens, Nüsslein-Volhard and Wieschaus 1984; Wieschaus, Nüsslein-Volhard and Jürgens 1984). Three, Pcl, thr and grh have been shown to cause defects in the embryonic cuticle (Nüsslein-Volhard, Wieschaus and Kluding 1984). The Pcl and thr alleles recovered here closely resemble previously described alleles. Prior to this screen grh was represented by only the one allele, with the possibility that the phenotype was atypical for null mutations at the locus. However three of the four grh alleles recovered here are virtually identical to the original grh allele in phenotype, with a degenerating head skeleton. They also appear to be more susceptible to rupture of the cuticle. The fourth allele, grh^{380}, is not embryonic lethal and thus appears to be hypomorphic.

The maternal component of the screen was less successful. We had originally planned to follow the general approach of Steward and Nüsslein-Volhard (1986), in using a dominant female sterile chromosome to ensure that only Df(2R)PC4/b pr cn wx^{wxl} bw females could lay eggs in the F_2. However we discovered that Df(2R)PC4/Fs(2)D flies tended to either die before eclosion or two show severe developmental and behavioral defects, and were unsuitable to use in the screen. This meant that screening for defective eggs had to be carried out in a background of normal oogenesis and embryogenesis, which mitigated against recovering mutations at any locus which produced either no eggs or a reduced number of eggs with not striking embryonic phenotype. For this reason we probably missed alleles at loci such as cay, sub, hal and fs(2)Ito. However the dramatic effects of stau mutations on segmentation patterns should have guaranteed their recovery and we cannot explain why none were recovered. Two novel maternal loci, designated fs(2)PC4 N and fs(2)PC4 T were identified. Only one allele was recovered for each, and although both complemented all the lethal loci mapping to their respective regions, it is possible that they represent hypomorphic alleles of undescribed lethal loci (c/f torpedo; Schüpbach 1987).

One question which we wanted to address was whether there are other loci in the region which play a similar role to that of those genes which affect embryonic development - stau, Pcl, thr and grh. The failure to recover stau alleles makes it difficult to argue that there are no stau like genes in the region. However the data for lethal loci is much more extensive and it is unlikely that other genes with functions similar to Pcl, thr or grh cluster to this region.

TABLE 1

Cytogenetics of chromosomal deficiencies

Deficiency	Breakpoints	Reference
Df(2R)PC4	55A;55C	This report
Df(2R)Pcl-XM82	(55A;55B)	This report
Df(2R)Pcl-7B	54E8-F1;55B9-C1	Duncan 1982
Df(2R)Pcl-11B	54F6-55A1;55C1-3	Duncan 1982
Df(2R)P66		?
Df(2R)P29		?
Df(2R)P34		?

TABLE 2

Loci mapping to the region defined by Df(2R)PC4

Locus	Alleles Recovered	Phenotype[a]	Previously Found[b]	Reference
Pcl	216, 372	E	Y	1
thr	313, 321, 454	E	Y	2
PC4-A	139, 458	LP	Y	3
PC4-B	110, 124, 378, 398, 399	LP	N	
stau	none	M	Y	4
eay	360, 455	M	Y	5
grh	300, 370, 380, 436	E	Y	2
PC4-C	197, 388	LP	N	
PC4-D	202, 414, 419	LP	N	
PC4-E	262, 322, 418, 420, 465	LP	N	
PC4-F	198, 463	LP	N	
PC4-G	51	E	N	
PC4-H	119	LP	N	
PC4-J	223	LP	N	
PC4-K	236	LP	N	
PC4-L	363	LP	N	
PC4-M	427	LP	N	
sub	397	M	Y	5
hal	none	M	Y	5
lto	none	M	Y	5
PC4-N	140	M	N	
PC4-P	41, 42	LP	N	
PC4-Q	267, 272	LP	N	
PC4-R	147, 459	LP	N	
PC4-S	159	E	N	
PC4-T	133	M	N	

References
1 Duncan (1982); Jürgens (1986)
2 Nüsslein-Volhard, Wieschaus and Kluding (1984)
3 Schüpbach pers. comm.
4 Schüpbach and Wieschaus (1986)
5 Schüpbach and Wieschaus (1988)

REGION 55 DATA

DEFICIENCIES

PC4	55A	55C
Pcl-XM82	?	?
Pcl-7B	54E8-F1	55B9-C1
Pcl-11B	54F6-55A1,2	55C1-3
Pcl-W5	?	?
Pu29	55C1,2	56B1,2
Pu34	55D2-E1	55E3,4
P66	55D2-E1	56B2

COMPLEMENTATION OF DEFICIENCIES

	XM82	7B	11B	Pu29	Pu34	P66
PC4	-	-	-	-	-	-
XM82	*	-	-	+	+	+
7B	*	*	-	+	+	+
11B	*	*	*	+	+	+
Pu29	*	*	*	*	-	-
Pu34	*	*	*	*	*	-

CLASSES

1= XM82$^-$ 7B$^-$ 11B$^-$

2= XM82$^-$ 7B$^-$ 11B$^+$

3= XM82$^-$ 7B$^+$ 11B$^+$

4a= XM82$^+$ 7B$^+$ 11B$^+$ Pu29$^-$ Pu34$^-$ P66$^+$

4b= XM82$^+$ 7B$^+$ 11B$^+$ Pu29$^-$ Pu34$^-$ P66$^-$

1) XM82⁻, 7B⁻, 11B⁻

Pcl 216, 372
thr 313, 321, 454
HD53 139, 458
IV 110, 124, 378, 398, 399
eay 360, 455

2) XM82⁻, 7B⁻, 11B⁺

grh 300, 370, 380, 436
II 197, 388
III 202, 414, 419

3) XM82⁻, 7B⁺, 11B⁺

I 262, 322, 418, 420, 465
II 198, 463
III 51
IV 119
V 223
VI 236
VII 363
VIII 427
sub 397
IX 140

4) XM82⁺, 7B⁺, 11B⁺

I 41, 42
II 267, 272
III 147, 459
IV 159
V 133