PART 1 - GENERAL INFORMATION

**Project Title:** Rearranging chromatin domains within the bithorax complex of hox genes in *Drosophila melanogaster*

**Keywords:** Homeotic genes, bithorax complex, chromatin and cis-regulatory elements

**Start Date:** January 14, 2010

**Duration:** 3 months

PART 2 - EXCHANGE PARTICIPANT(S) DETAILS

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3.1 Purpose of visit:

To exchange expertise for probing the co-linearity between the genomic organization of homeotic genes and body axis of the fruit fly Drosophila

3.2 Short description of work carried out during the visit:

Introduction:

The Drosophila bithorax complex (BX-C) is one of two homeotic gene clusters in the fly and is responsible for determining the segmental identity of the posterior thoracic segment and each of the fly abdominal segments [1,2]. It does this by using a >300 kb cis-regulatory region to control the parasegement-specific expression of the three BX-C homeotic genes: Ubx, abd-A and Abd-B (for review, see [3]). From the early genetic analysis of the BX-C to the most recent data involving enhancer trap lines, it was shown that its cis-regulatory sequences can be divided into nine parasegment-specific chromosomal domains (abx/bx, bxd/pbx, and iab-2 through iab-8), where each domain controls the activation of one of the three BX-C homeotic genes in a pattern appropriate for that parasegment [4,5,6,7,8]. Remarkably enough the parasegment-specific cis regulatory domains are aligned on the chromosome in the same order as the parasegments they specify along the antero-posterior axis of the fly (colinearity).

Through the many years of genetics of the BX-C by Ed Lewis, hundreds of mutations are available. Using imprecise excision of P-elements that had landed within the BX-C we have generated many internal deficiency alleles. Despite these intense mutagenesis efforts, internal inversions relocating only the parasegment-specific regulatory regions have never been recovered.

Aim:

In order to test for the importance of colinearity for proper BX-C regulation, we proposed to generate inversions within the BX-C by placing FLP/FRT yeast site-specific recombination sequences at specific locations. When two FRT sites inserted at different locations within the BXC are in trans with a source of Flippase, three types (deletion, tandem duplication or inversion) of chromosomes can be generated between the 2 sites (Figure 1).
Procedure:

We decided to manipulate three chromatin domains (iab4-iab5-iab6) of the BXC. In the proposal, we planned to take advantage of an existing chromosome with an FRT site placed near the Fab-4 boundary and to recombine it with another chromosome in which we planned to introduce an FRT site within the boundary Fab-7 by fC31 mediated integration [9; Cléard and Karch, in preparation]. While this scheme was allowing us to invert the order of the iab-4, iab-5 and iab-6 regulatory domains, we reasoned in retrospect that the whole procedure was too much work to finally end up with a single possibility of rearrangement. We thus decided to adapt our strategy to gain the flexibility of generating more rearrangements, deletions and/or integrations. Our new strategy is divided into 4 steps. The 1st step consists in generating a deletion of the iab-4, iab-5 and iab-6 domains and to replace them by an FC31 integration site (attP). In the 2nd step, we use recombineering to capture the corresponding iab-4 through iab-6 region in a BAC vector carrying an attB integration site and the yellow reporter gene that will allow us to score for integration within the BX-C. The 3rd step consists of integrating FRT sites at various locations within the BAC by recombineering. Finally in the 4th step the manipulated BACs are reintegrated within the chromosome platform generated in step 1. During my 3 months visit in Geneva I have achieved the construction of the integration vector (for step 1) as well as the construction of the iab-4-iab-6 BAC vector (step 3).

Results:

To generate the deletion from iab-4 through iab-6 we started from an integration platform that was constructed by Carole Iampietro, a former graduate student in the laboratory of François Karch (manuscript presently submitted). In this line (Δiab-6-attB) the whole iab-6 domain is deleted and replaced by an attP integration site (Fig.3). To widen the deletion towards iab-4 and create a new integration platform, we designed a targeting vector that has one attB site to target attP in Δiab-6-attB, 2 attP sites for subsequent targeting by cassette
exchange, a yellow gene as selection marker, 1 I-Sce1 site to make double strand break and an iab-3/Fab4 homology region (Fig.2).

Figure 2. Targeting vector

The construction of the targeting vector involved five steps of cloning and was achieved during my visit in Geneva. We are presently injecting the vector into the Δiab-6-attP platform. Once integrated (see Fig.3), the yellow* marker gene is regulated by the nearby iab-5 cis-regulatory domain, giving rise to flies with the 5th and more posterior abdominal segments pigmented while the more anterior segments remain yellow. The iab-3/Fab-4 homology region is included within the targeting vector to widen the iab-6 deletion to the edge of iab-4. Widening of the deletion towards iab-4 will be generated by creating a double strand break with I-Sce1 enzyme to activate homologous recombination of the flanking sequence. As we have provided iab-3/Fab-4 homology sequences within the vector it will lead to the deletion of iab-4 to iab-6 and leave the yellow marker gene flanked by 2 attP sites (Δiab-4,5,6-attP-yellow-attP; Fig.3). It should be noticed that the resulting recombination event will bring the yellow marker gene in the vicinity of iab-3 and should result in flies in which the pigmented segments should expand from the 3rd abdominal segment.
The presence of 2 attP allows will allow cassette exchange with targeting vectors carrying the manipulated iab-4 to iab-6 domains flanked by 2 attB sites; exchange being monitored by the loss of the yellow marker. The presence of attB and attP sites in cis on the targeting vector may interfere with integration frequency within the Δiab-6attP platform. It should be noticed however that Bischof et al have succeeded in integrating targeting constructs carrying an attP and attP sites next to one another in their split white strategy [9]. While we cannot predict the integration frequency with our setup, it should be noticed that a single event is enough for us to proceed further. We are prepared to scale up our injection protocol in order to recover this integration event.


3.3 Outcomes:
During this visit I prepared a vector that can be used to target the fly having attP site in BX-C. In parallel to the preparation of the targeting vector shown in Figure 2, I have learned recombineering technology to manipulate BAC (bacterial artificial chromosome) in the bacteria from Dr. Rob Maeda, a post doctoral fellow in the laboratory of François Karch. We first modified a targeting vector that is presently used in the laboratory of François Karch to...
introduce a 2nd attB site that will allow to perform cassette exchange. I then introduced flanking homology sequences to capture the iab-4,5 and 6 chromatin domains from a BX-C BAC using recombineering (data not shown). After retrieving the three-chromatin domains in this vector we plan to insert 2 FRT sites in opposite orientations at different location. The resulting constructs will then be integrated within the Δiab-4,5,6-attP-yellow-attP platform (see above) by cassette exchange, the integration event being scored by the loss of the yellow marker gene.

We have modified the initial proposal to obtain more flexibility to reorganize the iab-4, 5 and 6 regulatory domains. In effects, the experiments that can be foreseen once we obtain the Δiab-4,5,6-attP-yellow-attP platform go beyond the scope of my thesis program and I may not be able to exploit all the benefice of my efforts. I also wish to emphasize that the 3 months I spent in Geneva have strengthened my knowledge of the genetics of the bithorax complex.

3.4 Future collaboration with host institution

By generating this new tool I am providing the grounds for hopefully fruitful collaborations between the laboratories of Rakesh Mishra (my thesis adviser) and François Karch. We are together pursuing above mentioned work in future also. We may have to plan more visits like this for further discussion and exchange of experties.

3.5 Various comments:

Everything worked very well except obtaining working visa. I would like to suggest, if this visit can be made possible with visiting or a short-term visa.

3.6 Projected publications/articles resulting or to result from the exchange:

This work has high possibility of getting published in reputed journal, but it will take more time to get the expected results.