Chromosome structure and gene expression

The chromosomes of multicellular animals have a regular and inheritable physical organization. This was first recognized in studies dating back to the 1890's on the lampbrush chromosomes in amphibian oocytes (Fig.1) and the polytene chromosomes of insects. Subsequent studies have shown that the architectural principles inferred from analysis of lampbrush and polytene chromosomes are universal features of chromosomes throughout much of the animal kingdom. The key organizational principle is the subdivision of the chromatin fiber into a series of independent looped domains, called "TADs." The arrangement of TADs along the chromosome tend to be invariant and are largely independent of the cell type or developmental stage. This regular and inheritable organization is a reflection of the underlying mechanism of TAD formation. TADs are separated from each other by special elements called boundaries or insulators. While these elements have been found in many different species, they have been most fully characterized in *Drosophila*. Fly boundaries span DNA sequences of 150 bp to 1.5 kb in length and contain one or more nucleosome-free nuclease-hypersensitive regions. These nuclease-hypersensitive regions are targets for a large collection of DNA binding proteins that have been implicated in boundary function.

Boundary elements in flies are not only responsible for organizing the chromatin fiber, they also have genetic activities. When interposed between enhancers or silencers and target promoters, boundary elements block regulatory interactions This insulating activity provides a mechanism for delimiting units of independent gene activity: genes located between a pair of compatible boundaries are subject to regulatory interactions with enhancers/silencers present in the same chromosomal interval, while they are insulated from the effects of enhancers/silencers located beyond either boundary in adjacent regulatory neighborhoods. Genetic studies suggest that the insulating activity of boundary elements is a consequence of subdividing the chromosome into a series of topologically independent domains. Organizing the chromatin fiber into looped domains enhances contacts between sequences within the loop, while it suppresses contacts with sequences outside of the loop.

Our current research efforts are aimed at determining how boundaries function. A combination of genetic and molecular studies indicate that fly boundaries are functionally non-autonomous and that their activities in both loop formation and gene regulation depend upon their ability to engage in direct physical interactions with other boundaries. Physical interactions are mediated by the DNA binding proteins associated with potential pairing partners. Many fly chromosomal architectural proteins (Pita, Zipic, CTCF) form homomultimers. Consequently, if two boundaries share binding sites for one or more of these proteins, the two boundaries can be linked together by the multimer. In other cases, two different proteins can link two boundaries together by forming heteromeric complexes.

Boundary pairing interactions have several important properties which we are investigating. These include partner preferences and orientation dependence. The properties have important implications both for the formation of TADs, as well as TADs within TADs, and for long distance regulatory interactions.

Selected Publications:

1) The 87A7 chromomere. Identification of novel chromatin structures flanking the heat shock locus that may define the boundaries of higher order domains. Udvardy A, Maine

E, Schedl P.J Mol Biol. 1985 Sep 20;185(2):341-58. doi: 10.1016/0022-2836(85)90408-5.PMID: 2997449

2) A position-effect assay for boundaries of higher order **chromosomal** domains. Kellum R, Schedl P. Cell. 1991 Mar 8;64(5):941-50. doi: 10.1016/0092-8674(91)90318-s.PMID: 1848159

3) A group of scs elements function as domain boundaries in an enhancer-blocking assay. Kellum R, Schedl P.Mol Cell Biol. 1992 May;12(5):2424-31. doi: 10.1128/mcb.12.5.2424-2431.1992.PMID: 1569958

4) Fab-7 functions as a chromatin domain boundary to ensure proper segment specification by the Drosophila bithorax complex. Hagstrom K, Muller M, Schedl P.Genes Dev. 1996 Dec 15;10(24):3202-15. doi: 10.1101/gad.10.24.3202.PMID: 8985188

5) The mcp element from the Drosophila melanogaster bithorax complex mediates long-distance regulatory interactions. Muller M, Hagstrom K, Gyurkovics H, Pirrotta V, Schedl P.Genetics. 1999 Nov;153(3):1333-56. doi: 10.1093/genetics/153.3.1333.PMID: 10545463

6) Deletion of an insulator element by the mutation facet-strawberry in Drosophila melanogaster. Vazquez J, Schedl P.Genetics. 2000 Jul;155(3):1297-311. doi: 10.1093/genetics/155.3.1297.PMID: 10880489

7) Protein:protein interactions and the pairing of boundary elements in vivo. Blanton J, Gaszner M, Schedl P.Genes Dev. 2003 Mar 1;17(5):664-75. doi: 10.1101/gad.1052003.PMID: 12629048

8) Mechanism of **chromosomal** boundary action: roadblock, sink, or loop? Gohl D, Aoki T, Blanton J, Shanower G, Kappes G, Schedl P.Genetics. 2011 Mar;187(3):731-48. doi: 10.1534/genetics.110.123752. Epub 2010 Dec 31.PMID: 21196526

9) Elba, a novel developmentally regulated chromatin boundary factor is a hetero-tripartite DNA binding complex. Aoki T, Sarkeshik A, Yates J, Schedl P.Elife. 2012 Dec 13;1:e00171. doi: 10.7554/eLife.00171.PMID: 23240086

10) Determinants of **Chromosome** Architecture: Insulator Pairing in cis and in trans. Fujioka M, Mistry H, Schedl P, Jaynes JB.PLoS Genet. 2016 Feb 24;12(2):e1005889. doi: 10.1371/journal.pgen.1005889. eCollection 2016 Feb.PMID: 26910731

11). Functional Dissection of the Blocking and Bypass Activities of the Fab-8 Boundary in the Drosophila Bithorax Complex. Kyrchanova O, Mogila V, Wolle D, Deshpande G, Parshikov A, Cléard F, Karch F, Schedl P, Georgiev P.PLoS Genet. 2016 Jul 18;12(7):e1006188. doi: 10.1371/journal.pgen.1006188. eCollection 2016 Jul.PMID: 27428541

12) The bithorax complex iab-7 Polycomb response element has a novel role in the functioning of the Fab-7 chromatin boundary. Kyrchanova O, Kurbidaeva A, Sabirov M, Postika N, Wolle D, Aoki T, Maksimenko O, Mogila V, Schedl P, Georgiev P.PLoS Genet. 2018 Aug 15;14(8):e1007442. doi: 10.1371/journal.pgen.1007442. eCollection 2018 Aug.PMID: 30110328

13) Boundaries mediate long-distance interactions between enhancers and promoters in the Drosophila Bithorax complex. Postika N, Metzler M, Affolter M, Müller M, Schedl P, Georgiev P, Kyrchanova O.PLoS Genet. 2018 Dec 12;14(12):e1007702. doi: 10.1371/journal.pgen.1007702. eCollection 2018 Dec.PMID: 30540750

14) Functional dissection of the developmentally restricted BEN domain chromatin boundary factor Insensitive. Fedotova A, Clendinen C, Bonchuk A, Mogila V, Aoki T, Georgiev P, Schedl P.Epigenetics Chromatin. 2019 Jan 3;12(1):2. doi: 10.1186/s13072-018-0249-2.PMID: 30602385

15) The insulator functions of the *Drosophila* polydactyl C2H2 zinc finger protein CTCF: Necessity versus sufficiency. Kyrchanova O, Maksimenko O, Ibragimov A, Sokolov V, Postika N, Lukyanova M, **Schedl P**, Georgiev P.Sci Adv. 2020 Mar 25;6(13):eaaz3152. doi: 10.1126/sciadv.aaz3152. eCollection 2020 Mar.PMID: 32232161

16). Boundaries potentiate polycomb response element-mediated silencing. Erokhin M, Gorbenko F, Lomaev D, Mazina MY, Mikhailova A, Garaev AK, Parshikov A, Vorobyeva NE, Georgiev P, **Schedl P**, Chetverina D.BMC Biol. 2021 Jun 2;19(1):113. doi: 10.1186/s12915-021-01047-8.PMID: 34078365

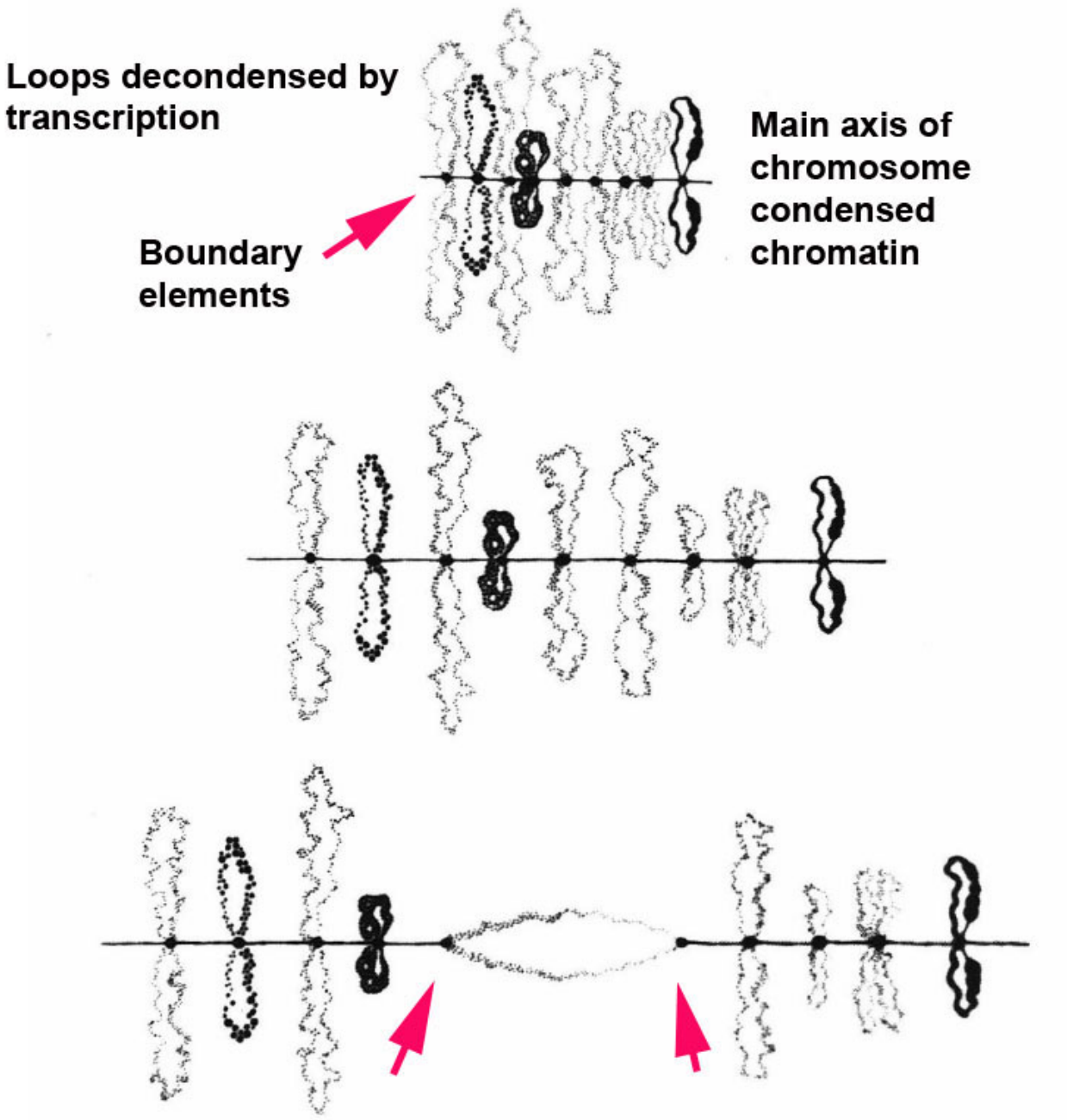

Fig. 1. Lampbrush chrosomoes. When lampbrush chromosomes are stretched pairing interactions between paired boundary elements can be broken. (Callan, 1963).

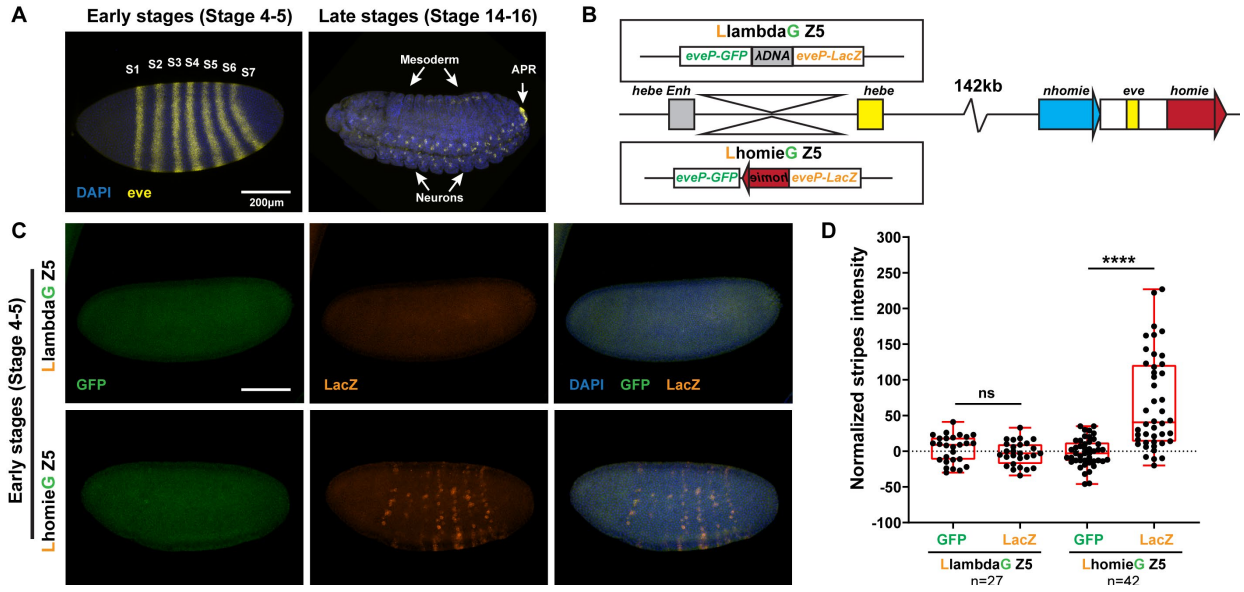

Fig. 2. Pairing interactions between a dual *lacZ-GFP* reporter (inserted at -142 kb) containing a *homie* boundary and the *homie* and *nhomie* boundaries in the *eve-skipped* (*eve*) locus can bring the transgene in close proximity to the *eve* locus. The *eve* enhancers can then activate expression of the reporter in early embryos; however, because boundary:boundary pairing interactions are orientation dependent, only one reporter (in this case *lacZ*) is activated.

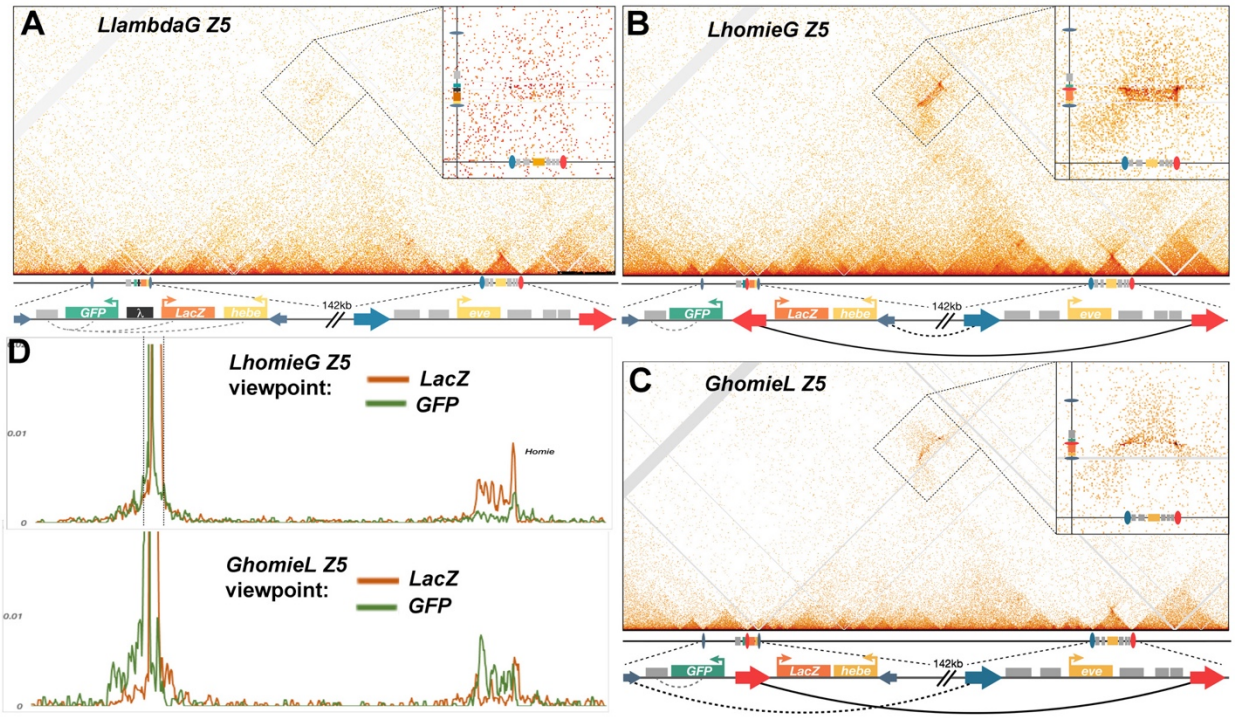

Fig. 3. MicroC of the *eve* locus and the transgene insertion site at -142 kb. *Small dark blue arrows* – insulators up and downstream of *hebe* that appear important to demarcate interaction domains between –142kb and *eve*. *Large blue arrow* - endogenous nhomie. *Large red arrow* – *homie*, either endogenous or in the transgene. The direction of arrows follow established convention on *nhomie/homie*, and do not reflect orientation of insulator protein binding motifs *per se*. *Gray boxes* – enhancers. **A)** microC map of the control line *LlambdaG Z5*. Scaled cartoons of the two loci of interest are shown directly below the microC map, and an unscaled blowup of the elements of interest at each locus is provided. Within the microC map of the entire locus, a zoom-in of the off-diagonal interaction between –142kb insertion cassette and the endogenous *eve* locus is shown. Note a slight increase in interaction frequency (compare to Figure 1A). **B, C)** microC map of *LhomieG Z5* and *GhomieL Z5*, respectively. The only difference between the two lines is the orientation of the transgenic *homie*, as indicate below each Micro-C map. Blow up of the off-diagonal interaction between –142kb and *eve*, including scaled cartoons denoting features of interest on the top right corner. Note the changed pattern of interaction with endogenous *eve* locus due to the orientation switch. **D)** "Virtual 4C" maps obtained from microC maps of **"B"** on top, and **"C"** bottom panels, respectively. The viewpoints are shown from either the *lacZ* gene in orange or the *GFP* gene in green for both panels. Note the increased interaction of either gene with endogenous *eve* depending on the change of orientation of *homie* in each cassette.