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DE FRANCE
—1530—

Collège de France; Chaire:
Evolution du Développement et des Génomes
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Cours 2025: Régulation des Gènes du Développement: Les Séquences 'Enhancers'

Leçon 5, vendredi 21 mars 2025



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Régulation des Gènes du Développement

Comment les enhancers fonctionnent à grandes distances?

Topologie des paysages d'enhancers

- A. Les holo-enhancers
- B. Les super-enhancers
- C. Les archipels de régulation
- D. Les paysages de régulations pleiotropiques
(non exclusifs...)

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PROBLEMS & PARADIGMS
Prospects & Overviews

BioEssays

Understanding fundamental principles of enhancer biology at a model locus

Analysing the structure and function of an enhancer cluster at the α -globin locus

Mira Kassouf | Seren Ford | Joseph Blayney | Doug Higgs

Approches fonctionnelles du 'super-enhancer'

*Délétions variées

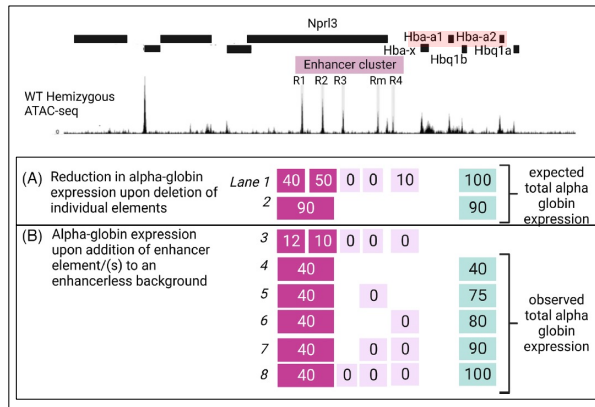
*Rajouts (sur une délétion totale..)

*Les enhancers n'ont pas la même importance.

*Différence dans le résultat selon que les enhancers sont enlevés ou rajoutés... en particulier sur l'importance de R3, Rm et R4.

*En effet, peu importants lorsqu'ils sont enlevés par unité, ils se révèlent critiques lorsque rajoutés à R1 et R2 (comparer les lignes 2 et 3 avec la ligne 8..).

*Importance de la topologie des enhancers au sein du SE, pour induire une architecture de régulation



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PROBLEMS & PARADIGMS
Prospects & Overviews

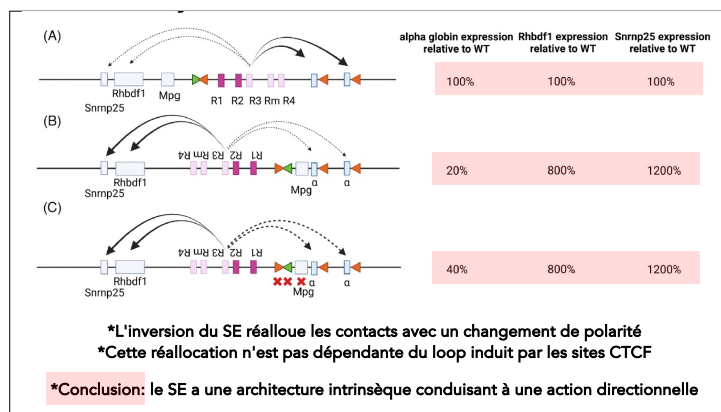
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La polarité du Super-Enhancer est importante



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Régulation des Gènes du Développement

*Thalassémies (Thalassa –la mer–)

***Alpha-thalassémies** (assez rare, Afrique, Arabie Saoudite, Inde...). Différents nombres d'allèles peuvent être touchés, de façon différente.. Le quadruple mutant est généralement très sévère, souvent létal.

***Bêta-thalassémies** (au minimum 350 mutations décrites, 60'000 naissances chaque année..). Fortes anémies, splénomégalie, malformations...

Prévalence augmentée dans les régions à haut taux de paludisme car la polyglobulie 'protège' en quelques sortes contre la malaria (les crises sont moins souvent mortelles..). Une crise de paludisme entraîne généralement une lyse de 30 à 50% des globules rouges (situation pouvant être létale). L'excès de globules rouges dans les thalassémies rend ces crises moins aiguës.

Les mutations touchent toutes les étapes de la synthèse des globines (régulation, RNA processing, gènes, traduction, assemblage des globines, stabilité...) et on estime que app 5% population en porte une. Les mutations dans les enhancers sont très rares et généralement familiales...

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NPRL3

HBZ

HBM

HBO1

HBA2

HBA1

R1

R2

R3

R4

*Délétions qui induisent des anémies

*R2 semble être particulièrement important

Consistent with these general observations, common natural variation of the human α -globin cluster causing α -thalassaemia is almost always due to deletions or nucleotide variants in the coding sequence. Nevertheless, deletions and duplications of the α -globin enhancer elements have been seen in sporadic families with α -thalassaemia (Figure 7), often from geographical regions where α -thalassaemia is otherwise rare. These have arisen by illegitimate recombination, telomeric truncation and translocation of the enhancers. These rare families provided some of the first examples of human enhanceropathies caused by deletion of enhancers and first pointed to the existence of distal regulatory elements controlling α -globin gene expression.

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Comment les enhancers fonctionnent à grandes distances?

*Au niveau moléculaire?

*Au niveau du 'choix' des gènes cibles et de l'organisation chromosomique?

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Les 'archipels de régulation'

A Regulatory Archipelago Controls *Hox* Genes Transcription in Digits

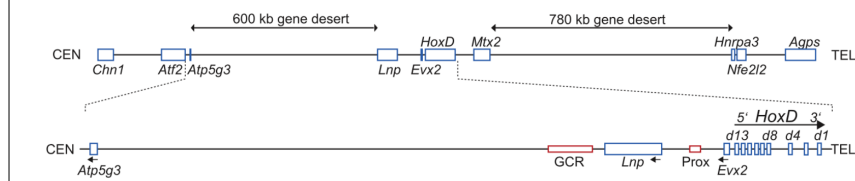
Thomas Montavon,¹ Natalia Sosnikova,² Bénédicte Mascroz,² Elisabeth Joye,¹ Laurie Thevenet,² Erik Splinter,² Wouter de Laat,² François Spitz,² and Denis Duboule^{1,2,*}

Cell 147, 1132–1145, November 23, 2011 ©2011 Elsevier Inc.

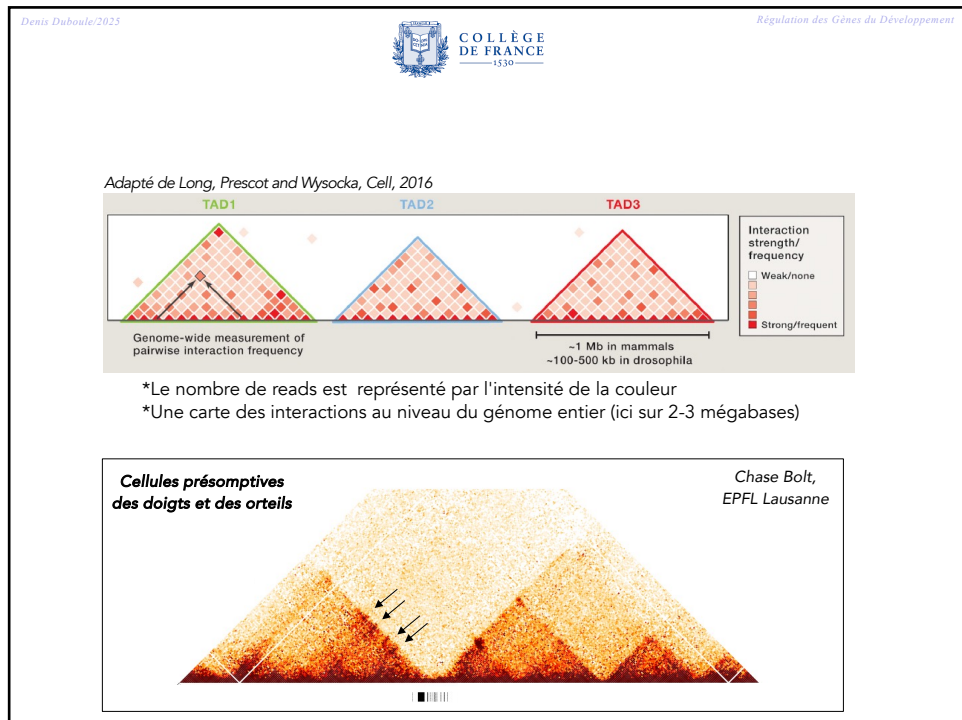
C. Les archipels de régulation

Transcription of *Hoxd* genes in digits integrates the collective activities of several regulatory elements. While some of these sequences did activate transcription in digits on their own, others may play a more structural role. 'Regulatory archipelagos' such as the one we characterize here may be numerous in vertebrate genomes and are different from other reported large scale regulatory controls at work e.g., at the β -globin locus, where various elements span a much shorter chromosomal segment (about 130 kb) and do not overlap with a gene desert (Tolhuis et al., 2002), or at the *Shh* locus, where activation in limb buds requires the association with a single enhancer, whose deletion abolishes transcription (Saga et al., 2005; Amano et al., 2009).

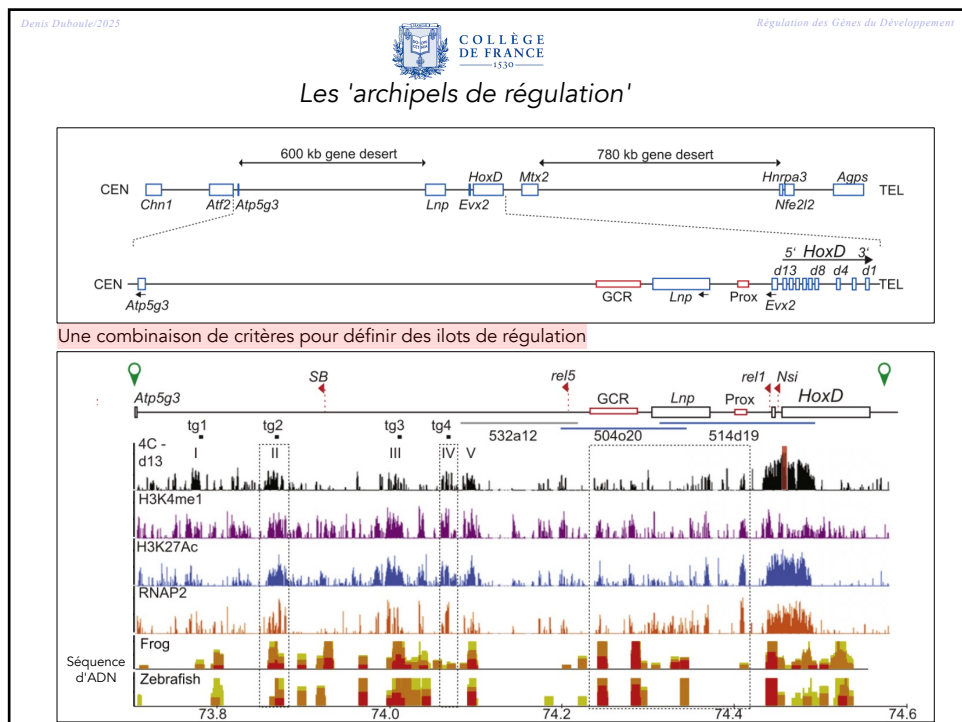
Le cluster de gènes *HoxD*: Plusieurs gènes cibles au milieu de deux grands déserts de gènes



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Régulation des Gènes du Développement

Les 'archipels de régulation'

A Regulatory Archipelago Controls *Hox* Genes Transcription in Digits

Thomas Montavon,¹ Natalia Soshnikova,² Bénédicte Mascroz,² Elisabeth Joye,¹ Laurie Thevenet,² Erik Splinter,³ Wouter de Laat,² François Spitz,¹ and Denis Duboule^{1,2,4}

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Contrairement à l'holo-enhancer (ex: *Fgf8*), les séquences distribuées dans le paysage sont complémentaires, plus que quantitativement additives. Leurs variations produiront donc des effets généralement qualitatifs plus que quantitatifs.

Figure 7. A Regulatory Archipelago Controls *Hoxd* Gene Expression in Digits

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Le gène *Shox2*

Le gène humain *SHOX* est localisé dans la région pseudo-autosomale 1 (sur le X et le Y, qui donc échappe à l'inactivation du X. Ce gène n'existe pas chez la souris, qui en revanche à *Shox1* et *Shox2*....

Les régions pseudo-autosomales (ou pseudo-autosomales, ou PAR pour Pseudo-Autosomal Region en anglais) sont des portions des chromosomes sexuels (ou gonosomes) homologues entre les deux chromosomes sexuels. Ainsi, elles sont héritées d'un point de vue statistique comme s'ils étaient portés par les autosomes.

Wikipedia
https://fr.wikipedia.org/wiki/R%C3%A9gion_pseudo-autosomale

PNAS

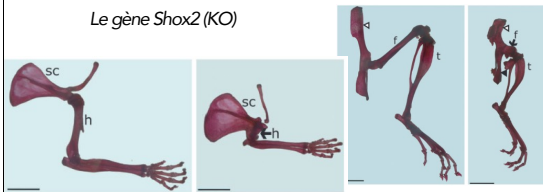
A mouse model for human short-stature syndromes identifies *Shox2* as an upstream regulator of *Runx2* during long-bone development

John Cobb¹, André Dierich², Yolande Huss-Garcia³, and Denis Duboule^{1*}

¹Department of Biology and Animal Biology and National Research Center "Frontiers in Genetics," Sciences B, University of Geneva, Quai Ernest Ansermet 36, 1211 Geneva 4, Switzerland; and ²Institut de Génétique et de Biologie Moléculaire et Cellulaire, Institut Clinique de la Souris, Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/Université Louis Pasteur/Collège de France, BP 107162, 67404 Strasbourg, France

Deficiencies or mutations in the human pseudoautosomal *SHOX* gene are associated with a series of short-stature conditions, including Turner syndrome, Leri-Weill dyschondrosteosis, and Langer mesomelic dysplasia. Although this gene is absent from the mouse genome, the closely related paralogous gene *Shox2* displays a similar expression pattern in developing limbs. Here, we report that the conditional inactivation of *Shox2* in developing appendages leads to a strong phenotype, similar to the human conditions, although it affects a different proximodistal limb segment.

Le gène *Shox2* (KO)



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nature communications

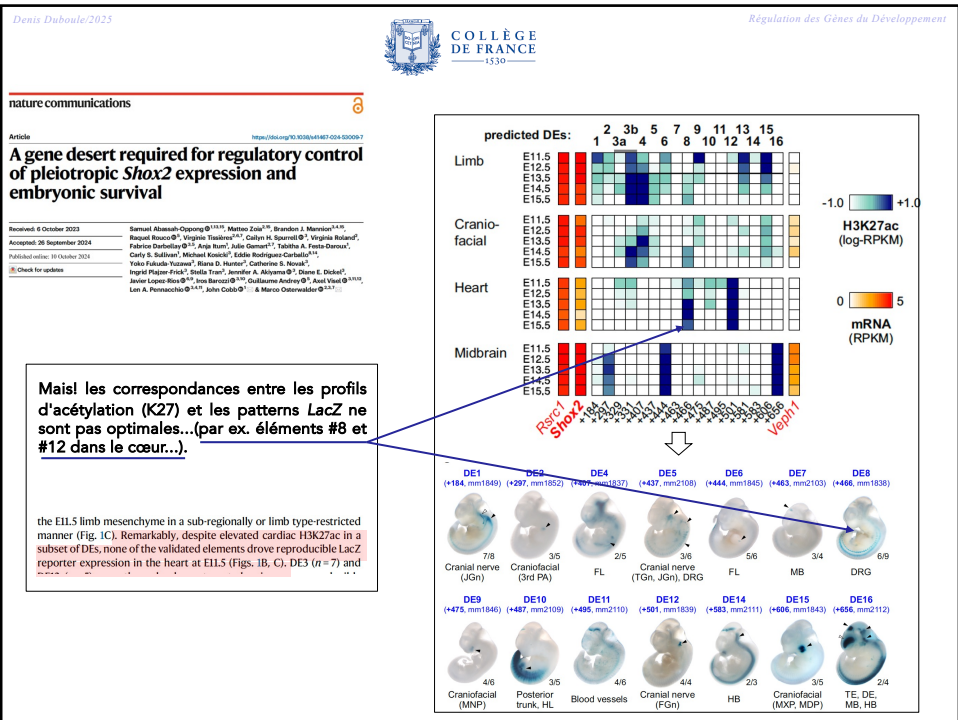
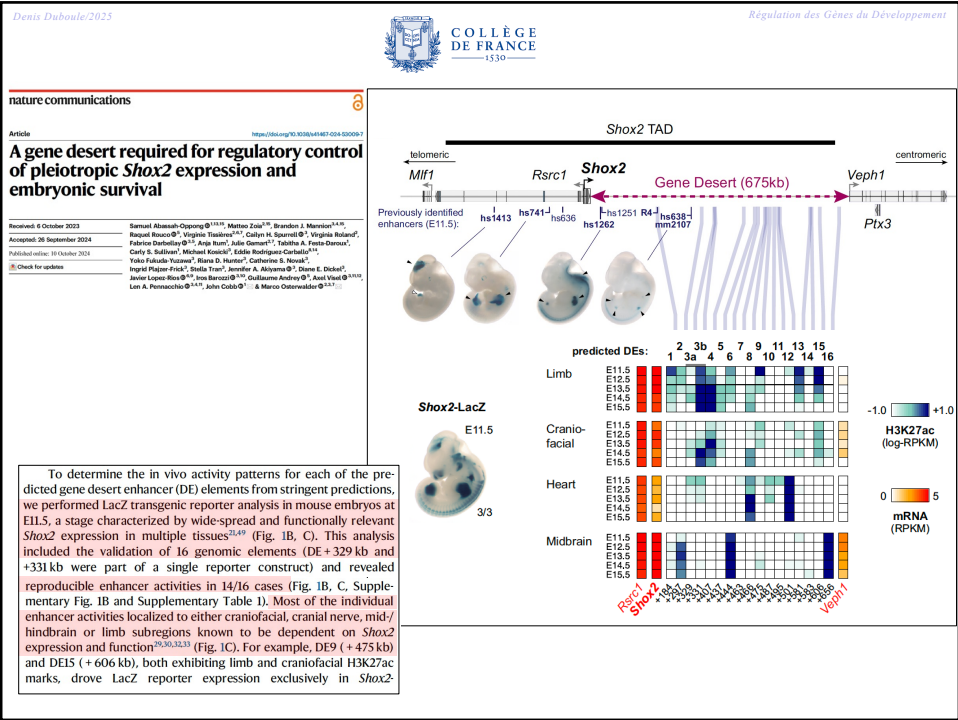
Article

<https://doi.org/10.1038/s41467-024-5009-7>

A gene desert required for regulatory control of pleiotropic *Shox2* expression and embryonic survival

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Samuel Abasah-Opong^{1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101,102,103,104,105,106,107,108,109,110,111,112,113,114,115,116,117,118,119,120,121,122,123,124,125,126,127,128,129,130,131,132,133,134,135,136,137,138,139,140,141,142,143,144,145,146,147,148,149,150,151,152,153,154,155,156,157,158,159,160,161,162,163,164,165,166,167,168,169,170,171,172,173,174,175,176,177,178,179,180,181,182,183,184,185,186,187,188,189,190,191,192,193,194,195,196,197,198,199,200,201,202,203,204,205,206,207,208,209,210,211,212,213,214,215,216,217,218,219,220,221,222,223,224,225,226,227,228,229,230,231,232,233,234,235,236,237,238,239,240,241,242,243,244,245,246,247,248,249,250,251,252,253,254,255,256,257,258,259,260,261,262,263,264,265,266,267,268,269,270,271,272,273,274,275,276,277,278,279,280,281,282,283,284,285,286,287,288,289,290,291,292,293,294,295,296,297,298,299,300,301,302,303,304,305,306,307,308,309,310,311,312,313,314,315,316,317,318,319,320,321,322,323,324,325,326,327,328,329,330,331,332,333,334,335,336,337,338,339,340,341,342,343,344,345,346,347,348,349,350,351,352,353,354,355,356,357,358,359,360,361,362,363,364,365,366,367,368,369,370,371,372,373,374,375,376,377,378,379,380,381,382,383,384,385,386,387,388,389,390,391,392,393,394,395,396,397,398,399,400,401,402,403,404,405,406,407,408,409,410,411,412,413,414,415,416,417,418,419,420,421,422,423,424,425,426,427,428,429,430,431,432,433,434,435,436,437,438,439,440,441,442,443,444,445,446,447,448,449,450,451,452,453,454,455,456,457,458,459,460,461,462,463,464,465,466,467,468,469,470,471,472,473,474,475,476,477,478,479,480,481,482,483,484,485,486,487,488,489,490,491,492,493,494,495,496,497,498,499,500,501,502,503,504,505,506,507,508,509,510,511,512,513,514,515,516,517,518,519,520,521,522,523,524,525,526,527,528,529,530,531,532,533,534,535,536,537,538,539,540,541,542,543,544,545,546,547,548,549,550,551,552,553,554,555,556,557,558,559,560,561,562,563,564,565,566,567,568,569,570,571,572,573,574,575,576,577,578,579,580,581,582,583,584,585,586,587,588,589,590,591,592,593,594,595,596,597,598,599,600,601,602,603,604,605,606,607,608,609,610,611,612,613,614,615,616,617,618,619,620,621,622,623,624,625,626,627,628,629,630,631,632,633,634,635,636,637,638,639,640,641,642,643,644,645,646,647,648,649,650,651,652,653,654,655,656,657,658,659,660,661,662,663,664,665,666,667,668,669,670,671,672,673,674,675,676,677,678,679,680,681,682,683,684,685,686,687,688,689,690,691,692,693,694,695,696,697,698,699,700,701,702,703,704,705,706,707,708,709,710,711,712,713,714,715,716,717,718,719,720,721,722,723,724,725,726,727,728,729,730,731,732,733,734,735,736,737,738,739,740,741,742,743,744,745,746,747,748,749,750,751,752,753,754,755,756,757,758,759,760,761,762,763,764,765,766,767,768,769,770,771,772,773,774,775,776,777,778,779,780,781,782,783,784,785,786,787,788,789,790,791,792,793,794,795,796,797,798,799,800,801,802,803,804,805,806,807,808,809,810,811,812,813,814,815,816,817,818,819,820,821,822,823,824,825,826,827,828,829,830,831,832,833,834,835,836,837,838,839,840,841,842,843,844,845,846,847,848,849,850,851,852,853,854,855,856,857,858,859,860,861,862,863,864,865,866,867,868,869,870,871,872,873,874,875,876,877,878,879,880,881,882,883,884,885,886,887,888,889,890,891,892,893,894,895,896,897,898,899,900,901,902,903,904,905,906,907,908,909,910,911,912,913,914,915,916,917,918,919,920,921,922,923,924,925,926,927,928,929,930,931,932,933,934,935,936,937,938,939,940,941,942,943,944,945,946,947,948,949,950,951,952,953,954,955,956,957,958,959,960,961,962,963,964,965,966,967,968,969,970,971,972,973,974,975,976,977,978,979,980,981,982,983,984,985,986,987,988,989,990,991,992,993,994,995,996,997,998,999,1000,1001,1002,1003,1004,1005,1006,1007,1008,1009,1010,1011,1012,1013,1014,1015,1016,1017,1018,1019,1020,1021,1022,1023,1024,1025,1026,1027,1028,1029,1030,1031,1032,1033,1034,1035,1036,1037,1038,1039,1040,1041,1042,1043,1044,1045,1046,1047,1048,1049,1050,1051,1052,1053,1054,1055,1056,1057,1058,1059,1060,1061,1062,1063,1064,1065,1066,1067,1068,1069,1070,1071,1072,1073,1074,1075,1076,1077,1078,1079,1080,1081,1082,1083,1084,1085,1086,1087,1088,1089,1090,1091,1092,1093,1094,1095,1096,1097,1098,1099,1100,1101,1102,1103,1104,1105,1106,1107,1108,1109,1110,1111,1112,1113,1114,1115,1116,1117,1118,1119,1120,1121,1122,1123,1124,1125,1126,1127,1128,1129,1130,1131,1132,1133,1134,1135,1136,1137,1138,1139,1140,1141,1142,1143,1144,1145,1146,1147,1148,1149,1150,1151,1152,1153,1154,1155,1156,1157,1158,1159,1160,1161,1162,1163,1164,1165,1166,1167,1168,1169,1170,1171,1172,1173,1174,1175,1176,1177,1178,1179,1180,1181,1182,1183,1184,1185,1186,1187,1188,1189,1190,1191,1192,1193,1194,1195,1196,1197,1198,1199,1200,1201,1202,1203,1204,1205,1206,1207,1208,1209,1210,1211,1212,1213,1214,1215,1216,1217,1218,121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Régulation des Gènes du Développement

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Figure 1: Shox2 TAD and its role in limb bud development.

The top panel illustrates the Shox2 TAD structure. A CTCF loop (indicated by a grey bar) connects the *Mir1* locus to the *Shox2* locus. The *Shox2* gene is located within a TAD that also contains the *Gene Desert* and the *HCD* (Homeo-Cluster Domain). The *Shox2* gene is flanked by *Rsrc1* and *Ptx3*. The *Shox2* gene is transcribed from the *Shox2* promoter (indicated by a red arrow) and contains several exons (1-16) and introns (1-15). The *Shox2* gene is expressed in the limb bud (indicated by a red arrow).

The middle panel shows a schematic of the Shox2 gene and its expression in the limb bud. The *Shox2* gene is transcribed from the *Shox2* promoter (indicated by a red arrow) and contains several exons (1-16) and introns (1-15). The *Shox2* gene is expressed in the limb bud (indicated by a red arrow).

The bottom panel shows a table of normalized expression levels for *Shox2* and *Rsrc1* in WT and *GD^Δ* limb buds. The table is divided into two sections: **FL (E10.5)** and **HL (E11.5)**. The *Shox2* section shows normalized expression levels for *Shox2* and *Rsrc1* in WT and *GD^Δ* limb buds. The *Rsrc1* section shows normalized expression levels for *Rsrc1* in WT and *GD^Δ* limb buds.

Gene	Genotype	Normalized expression
<i>Shox2</i>	WT	1.0
	<i>GD^Δ</i>	~0.5
	WT	1.0
	<i>GD^Δ</i>	~0.5
<i>Rsrc1</i>	WT	1.0
	<i>GD^Δ</i>	~0.5
	WT	1.0
	<i>GD^Δ</i>	~0.5

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A gene desert required for regulatory control of pleiotropic *Shox2* expression and embryonic survival

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Approche fonctionnelle

*Délétion du désert 'D-dom'

*Analyse par WISH et par qPCR

Remarkably, *Shox2* expression in distinct craniofacial compartments was more severely affected by the loss of the gene desert (Fig. 3D, E).

Par contre, presque tout est perdu pour les dérivés de la face...
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Mais, en dépit de l'absence de détection d'enhancer 'cardiaque' dans ce désert, sa délétion induit une perte de mRNAs dans le sinus veineux (où se trouvent les progéniteurs du pacemaker SAN), suivie de la mort des fœtus à jour E12...

Régulations qui échappent à la détection !

SAN: Sino-Atrial Node: Nœud Sinu-Atrial (sinusal...), qui induit une activité électrique rythmique.

Despite the lack of identification of any *in vivo* heart enhancers in the gene desert following transgenic reporter analysis from mRNA analysis in GD^{ΔA} embryos revealed absence of *Shox2* transcripts from the cardiac sinus venosus (SV) that harbors the population of SAN pacemaker progenitors⁵⁵ (Fig. 4A, B). In accordance with the essential role of *Shox2* in the differentiation of SAN progenitors and the related lethality pattern in *Shox2*-deficient mouse embryos³⁶, cardiac *Shox2* depletion in GD^{ΔA} embryos triggered arrested development and embryonic lethality at around E12 (*n* = 5/5) (Supplementary Fig. 4C).

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Régulation des Gènes du Développement

nature communications

Article

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Régulations qui échappent à la détection ⚠

SAN: Sino-Atrial Node: Nœud Sinu-Atrial (sinusal...), qui induit une activité électrique rythmique.

*Production d'un nouveau dataset ATACseq de cœur à 11.5
*Comparaison avec d'autres datasets disponibles dans la littérature
*Plusieurs sites nouveaux identifiés... testés par transgènes LacZ

*Un seul élément positif, à 325kb du promoteur de *Shox2*

(renversement de l'approche en situation négative...)

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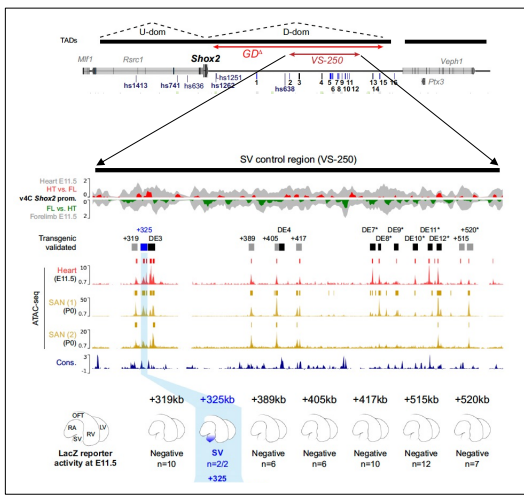
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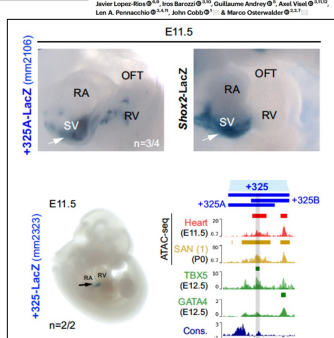
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The diagram illustrates the genomic context of the *Shox2* gene. It shows the gene structure with exons (numbered 1-14) and introns. Key regulatory elements include the U-domain, D-domain, and the SV control region (VS-250). A detailed view of the SV control region shows the v4C *Shox2* promoter, the SV control region (VS-250), and the SV control region (VS-250). The diagram also shows the expression of *Shox2* in the heart at E11.5, with the SV control region (VS-250) being the only element positive for LacZ reporter activity at E11.5.

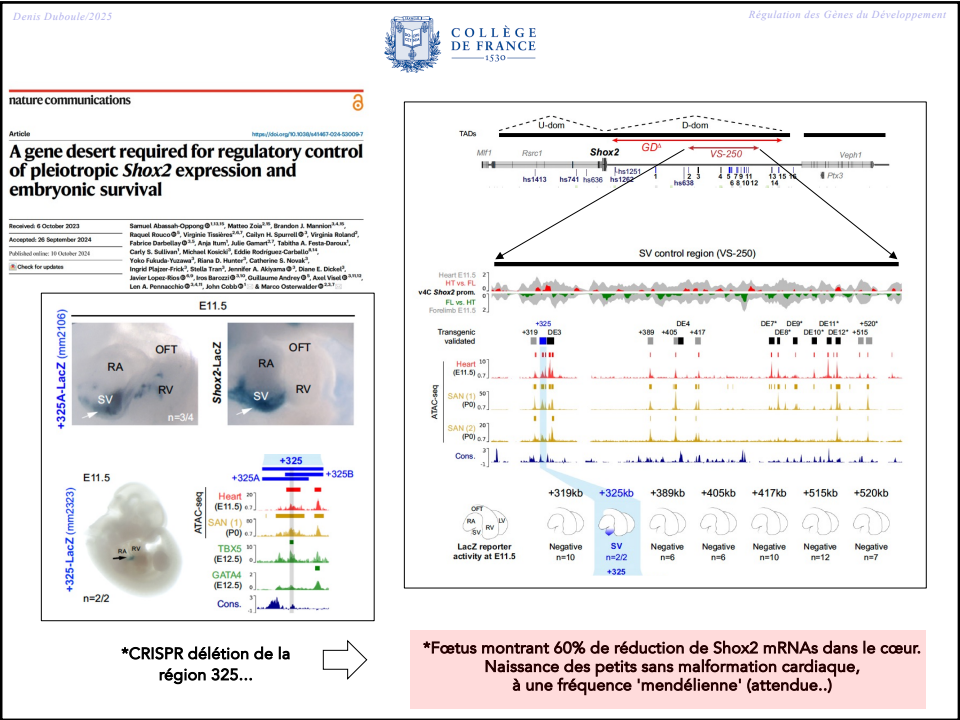


ATAC-seq and LacZ reporter activity data are shown for the SV control region (VS-250) at E11.5. The ATAC-seq tracks show peaks of accessibility at +325A and +325B. The LacZ reporter activity is shown for the SV control region (VS-250) at E11.5, with the SV control region (VS-250) being the only element positive for LacZ reporter activity at E11.5.

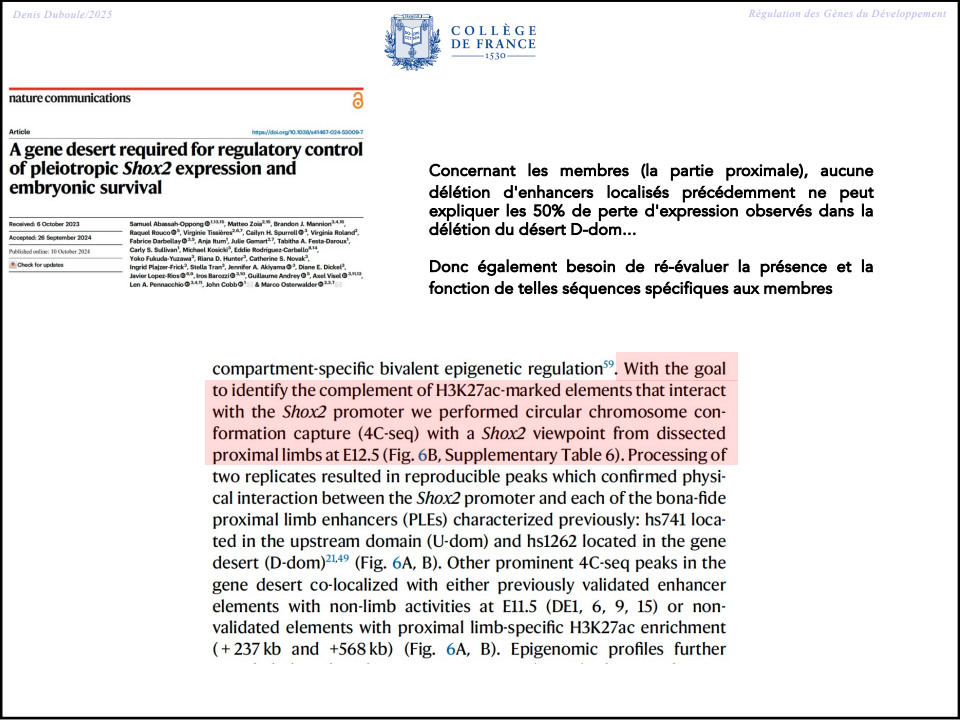
*Deux régions accessibles...

*Un seul élément positif, à 325kb du promoteur de *Shox2*

22



23



24

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*Approche en 4C, avec un point de vue fixé sur le gène cible *Shox2*. Les pics indiquent donc des contacts avec le gène *Shox2*.

*Cellules disséquées de la partie proximale du membre.

*Enhancers isolés précédemment utilisés comme contrôles...

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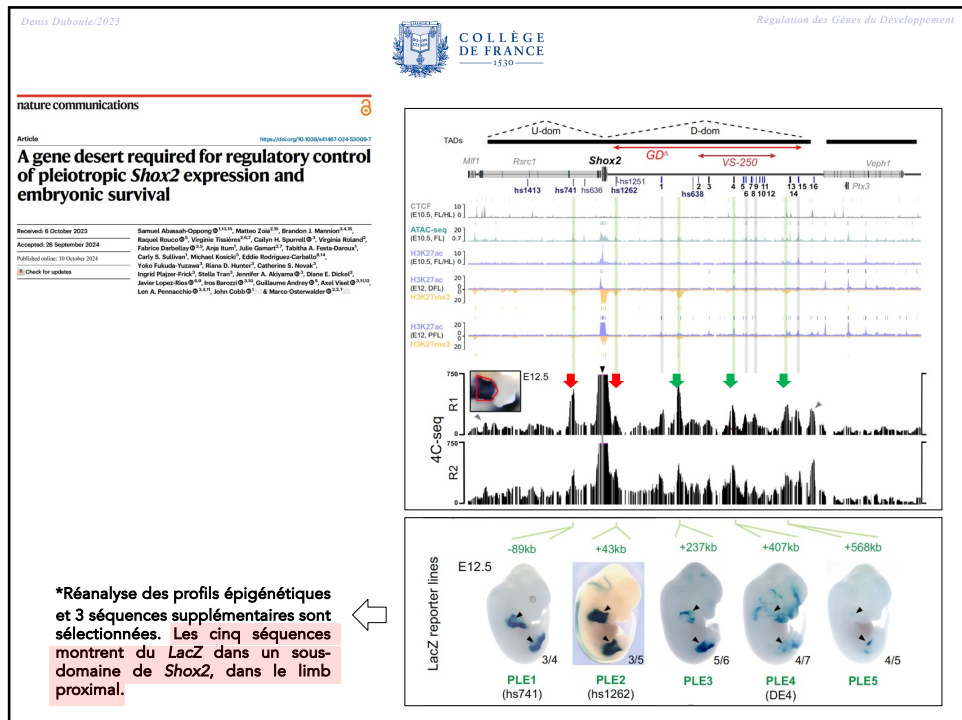
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*Réanalyse des profils épigénétiques et 3 séquences supplémentaires sont sélectionnées

*Approche semble bien fonctionner, avec des contacts clairs entre les deux enhancers et le gène *Shox2*.

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Lori A. Pennacchio^{37,38,39}, John Cado^{40,41} & Marco Osterwalder^{42,43,44}

Conclusions et points de discussion

*Remarques sur les problèmes d'approche expérimentale
*Des séquences H3K27Ac+ pas fonctionnelles
*Des séquences fonctionnelles sans marques 'classiques'

For example, recent studies evaluating H3K27ac-based tissue-specific enhancer predictions in mouse embryos revealed a substantial number of false-positives^{57,91}. In turn, a significant fraction (~14%) of validated in vivo enhancers were lacking enrichment of any canonical enhancer marks (ATAC-seq, H3K4me1, H3K27ac)⁹¹.

Gene desert functions

Proximal limb morphogenesis → Craniofacial patterning

SV/SAN pacemaker differentiation, embryonic viability

Shox2 gene desert

HCD (heart)

Shox2

PLE2, DE1, PLE3, DE2, +205 SV Enh, PLE4, DE3, DE4, DE5, DE6, DE7, DE8, DE9, DE10, DE11, DE12, PLE5, DE13, DE14, DE15, DE16

Limb Enh, Craniofacial Enh, Heart Enh, Cranial nerve/neuronal Enh

Gene desert (D-dom) chromatin conformation

Limb, Craniofacial, Heart

Tissue-invariant loop anchors, tissue-specific HCD

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Conclusions et points de discussion

*Remarques sur le modèle de régulation
*Les enhancers ne semblent pas être distribués selon une 'logique' (topologie) particulière.
*Ils pourraient cependant être impliqués dans la formation d'architectures chromatinienne différentes d'un tissu à l'autre, maximisant ainsi l'action globale sur le promoteur de *Shox2* (pleiotropie).
*Le domaine HCD en serait un exemple (pour 'mieux' positionner le 'heart enh').

Our findings support a model in which gene deserts provide a scaffold for preferential chromatin domains that generate enhancer-mediated cell type or tissue-specific *cis*-regulatory output based on the integration of upstream signals.

Gene desert functions

Proximal limb morphogenesis → Craniofacial patterning

SV/SAN pacemaker differentiation, embryonic viability

Shox2 gene desert

HCD (heart)

Shox2

PLE2, DE1, PLE3, DE2, +205 SV Enh, PLE4, DE3, DE4, DE5, DE6, DE7, DE8, DE9, DE10, DE11, DE12, PLE5, DE13, DE14, DE15, DE16

Limb Enh, Craniofacial Enh, Heart Enh, Cranial nerve/neuronal Enh

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Limb, Craniofacial, Heart

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Gene desert functions

Proximal limb morphogenesis

Craniofacial patterning

SV/SAN pacemaker differentiation, embryonic viability

Shox2 gene desert

HCD (heart)

PLE2, DE1, PLE3, DE2, +205 SV Enh, PLE4, DE5, DE6, DE7, DE8, DE9, DE10, DE11, DE12, PLE5, DE14, DE15, DE16

Shox2

Limb Enh, Heart Enh, Craniofacial Enh, Cranial nerve/neuronal Enh

Gene desert (D-dom) chromatin conformation

Limb, Craniofacial, Heart

HCD, VS-250

Tissue-invariant loop anchors, tissue-specific HCD

Conclusions et points de discussion

*Un modèle d'additivité qui semble être une solution souvent présente (archipèle).

The conventional model of enhancer additivity based on individual small and stable regulatory contributions is likely predominant in gene deserts⁹⁸. In support, we uncovered at least four *Shox2*-associated gene desert enhancers (PLE2-5) with overlapping activities in the proximal limb mesenchyme. Such regulatory architecture resembles the multipartite enhancer landscapes in *Indian Hedgehog* (*Ihh*) or *Gremlin1* loci, which as *Shox2* are involved in spatiotemporal coordination of proximal-distal limb identities with chondrogenic cues^{24,26}. Our study further reveals gene desert

31

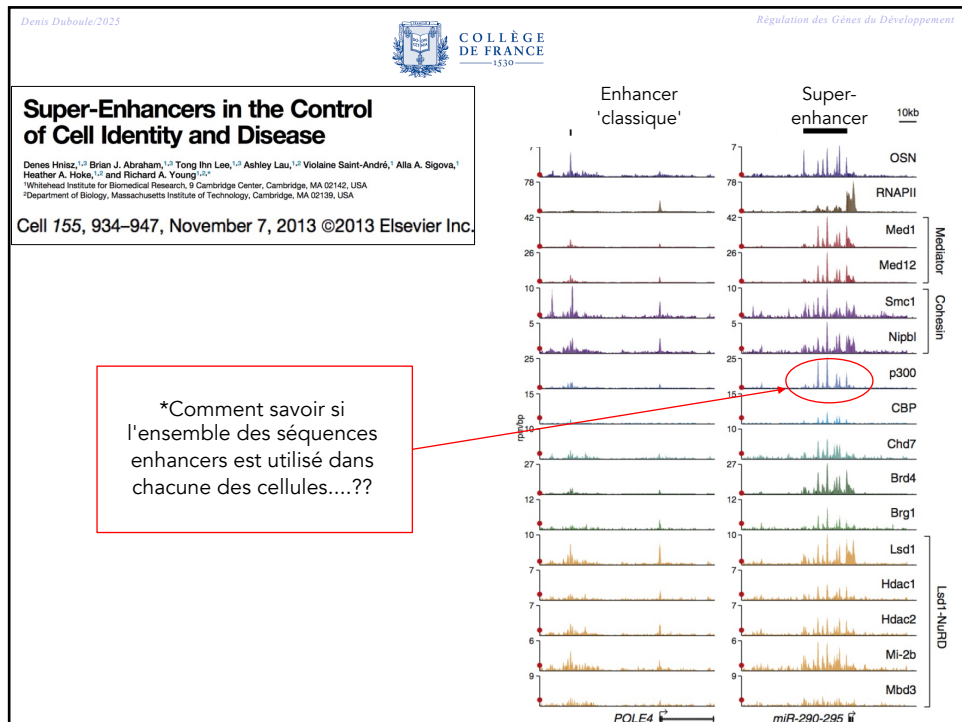
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Comment les enhancers fonctionnent à grandes distances?

*Toutes ces conclusions sont basées sur l'analyse simultanée de multiples situations (groupes de cellules..).

*Les fonctionnalités de ces situations ne sont peut-être pas égales et les configurations les plus fréquentes (donc les plus visibles) ne sont par nécessairement les plus productives.

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*Les fonctionnalités de ces situations ne sont peut-être pas égales et les configurations les plus fréquentes (donc les plus visibles) ne sont par nécessairement les plus productives.

*Comment analyser ces configurations chromatiniennes au niveau des cellules uniques?

34



Comment les enhancers fonctionnent à grandes distances?

*Comment analyser des configurations au niveau cellulaire?

Plusieurs approches sont possibles

*Configurations multiples au locus *Shh*

35



A long-range *Shh* enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly

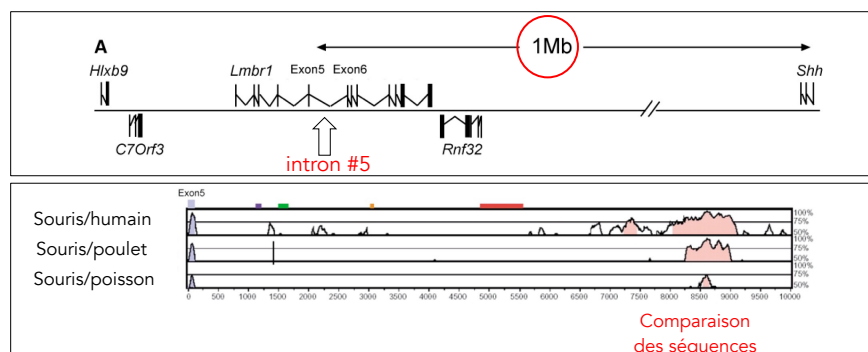
Laura A. Lettice¹, Simon J.H. Heaney¹, Lorna A. Purdie¹, Li Li², Philippe de Beer², Ben A. Oostra², Debbie Goode¹, Greg Elgar¹, Robert E. Hill^{1,*} and Esther de Graaf²

Human Molecular Genetics, 2003, Vol. 12, No. 14 1725–1735

RAPPEL

Le locus *Sonic Hedgehog (Shh)*

*L'analyse comparative des séquences d'ADN dans cet intron révèle la présence d'une région conservée jusqu'aux poissons

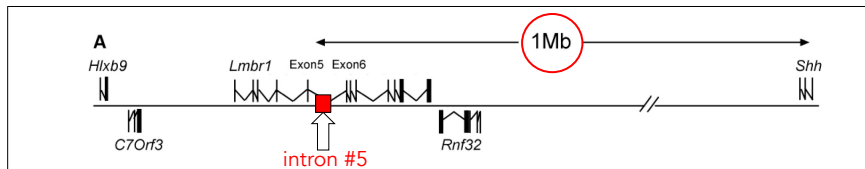
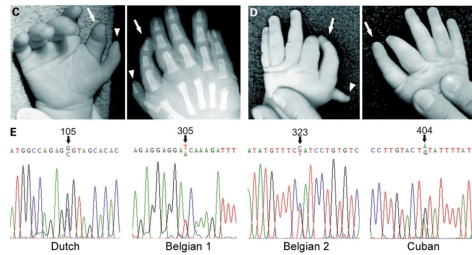


36

A long-range *Shh* enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly

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Polydactylie pré-axiale



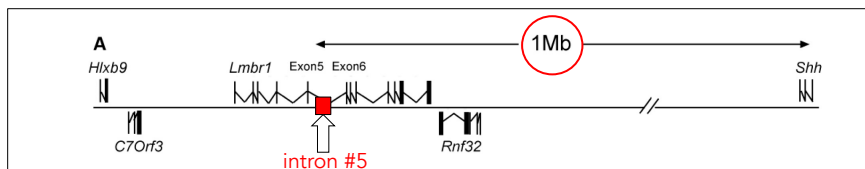
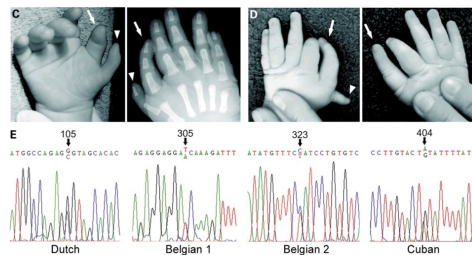
Les mutations semblent affecter l'expression de *Shh*.
 L'enhancer sera donc appelé : **ZRS** (ZPA Regulatory Sequence)

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A long-range *Shh* enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly

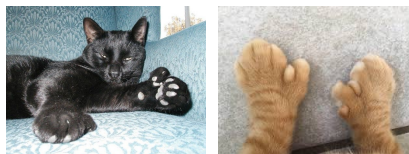
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Polydactylie pré-axiale



Des mutations qui sont aussi responsables de la polydactylie des chats (les chats de Hemingway)

https://fr.wikipedia.org/wiki/Chat_polydactyle



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cis-Regulatory Mutations Are a Genetic Cause of Human Limb Malformations

Julia E. VanderMeer and Nadav Ahituv*
Department of Bioengineering and Therapeutic Sciences, and Institute for Human Genetics,
University of California San Francisco, San Francisco, California

Dev Dyn. 2011 May; 240(5): 920-930. doi:10.1002/dvdy.22535.

Mutations humaines réintroduites dans la souris (transgènes)

Dans les deux cas (vraisemblablement dans toutes les mutations polydactyles impliquant la ZRS), l'expression de *Shh* est activée dans la partie antérieure du bourgeon de membre, comme si la ZRS avait normalement une capacité de régulation plus large que 'postérieure' mais que cette capacité était inhibée/réprimée ailleurs que dans les cellules de la ZPA.

Dans ce contexte, chaque mutation affectant la ZRS pourrait contribuer à la dérégpression (déstabilisation) de l'activité de cette séquence dans la partie antérieure

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© 2019. Published by The Company of Biologists Ltd | Development (2019) 148, doi:10.1242/dev.179623

RESEARCH ARTICLE

Developmentally regulated *Shh* expression is robust to TAD perturbations

Iain Williamson, Lauren Kane, Paul S. Devenney, Ilya M. Flyamer, Eve Anderson*, Fiona Kilanowski, Robert E. Hill, Wendy A. Bickmore* and Laura A. Lettice*

Délétion de 700 Kb entre la ZRS et le gène *Shh*

*Un rapprochement de 700 Kb entre la ZRS et son gène cible *Shh* ne change pas l'expression du gène de façon visible dans les membres, mais supprime l'expression dans le système nerveux

*Contrôles

*Del/Del

40

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Régulation des Gènes du Développement

DGD
Development, Growth & Differentiation

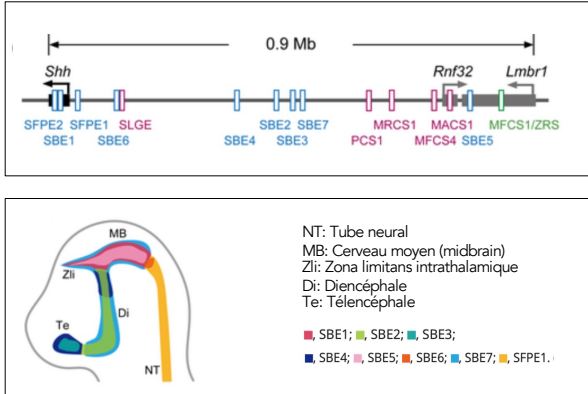


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REVIEW ARTICLE
Free Access

Gene regulatory landscape of the sonic hedgehog locus in embryonic development

Takanori Amano



NT: Tube neural
MB: Cerveau moyen (midbrain)
Zli: Zona limitans intrathalamique
Di: Diencéphale
Te: Télencéphale

■ SBE1; ■ SBE2; ■ SBE3;
■ SBE4; ■ SBE5; ■ SBE6; ■ SBE7; ■ SFPE1.

Enhancer membres
Enhancers gastro-intestinaux
Enhancers cerveau
Zli: Zona Limitans intrathalamica. Zone de signalisation (pendant le développement) importante pour le diencéphale, qui 'sépare' le thalamus ventral du thalamus dorsal.

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Régulation des Gènes du Développement

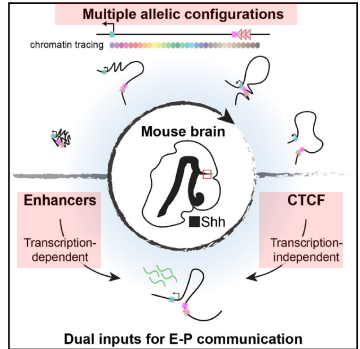




Article

Multiple allelic configurations govern long-range *Shh* enhancer-promoter communication in the embryonic forebrain

Jalynn Harke,^{1,2} Jeevan R. Lee,¹ Son C. Nguyen,^{1,2} Arjan Arab,^{1,3} Staci M. Rakowski,¹ Sewert Hugelier,² Christina Palosa,¹ Antonella Rikova,^{1,2} Rebecca Yumoto,^{1,2} Kellen Xu,¹ Yao Yao,¹ Melike Lakadamyali,^{1,2} Guillaume Andrey,^{1,2} Douglas J. Epstein,^{1,2} and Eric F. Joyce^{1,2,3}

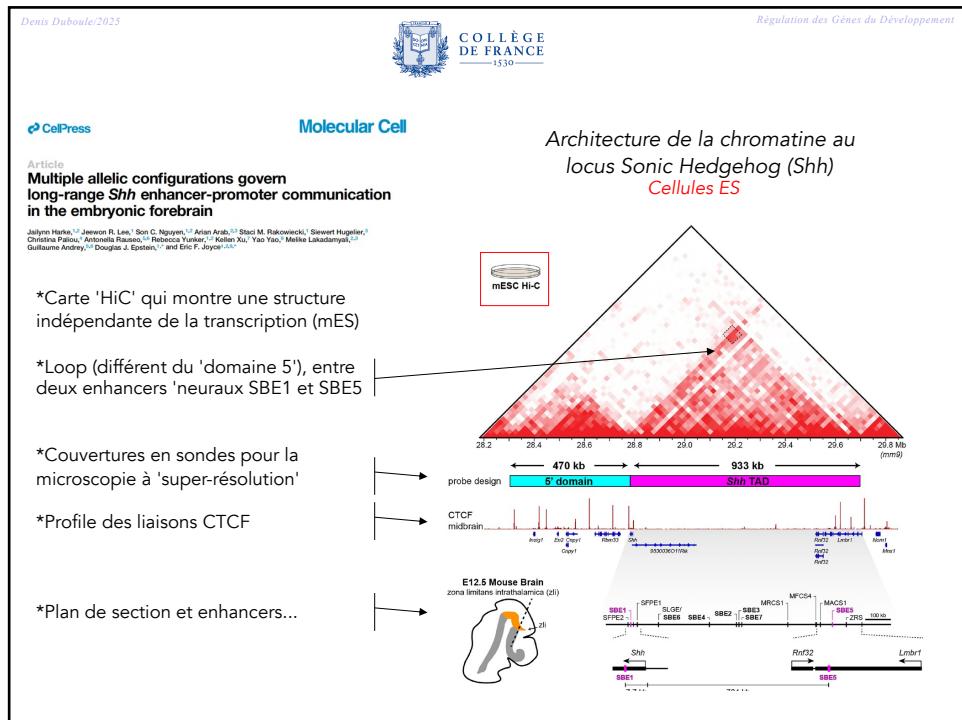


Multiple allelic configurations
chromatin tracing
Mouse brain
Shh
Enhancers
Transcription-dependent
CTCF
Transcription-independent
Dual inputs for E-P communication

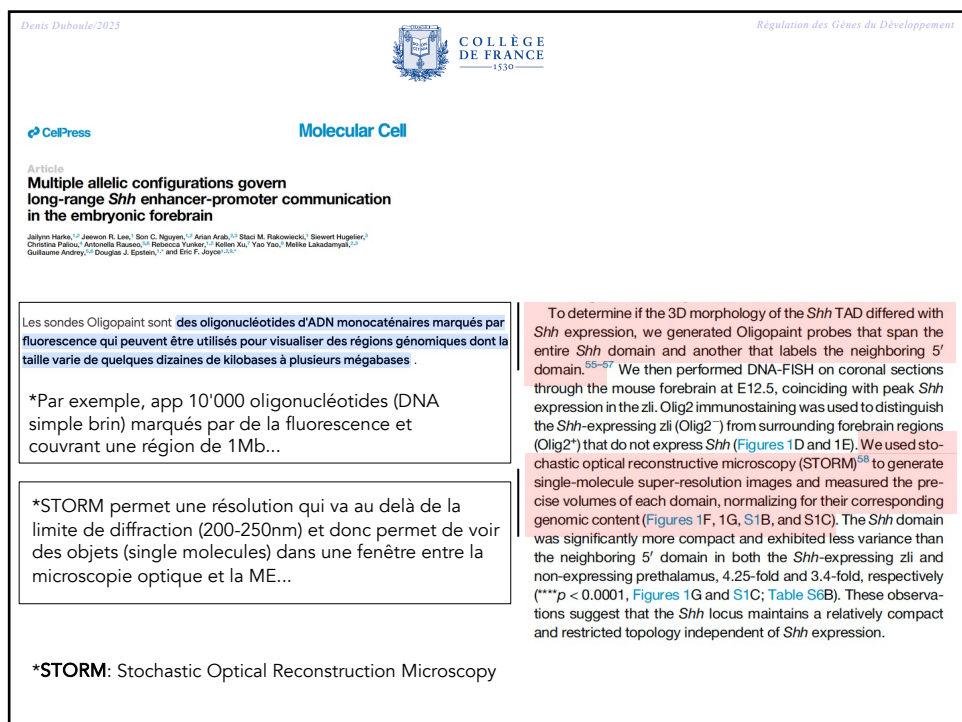
SUMMARY

Developmental gene regulation requires input from enhancers spread over large genomic distances. Our understanding of long-range enhancer-promoter (E-P) communication, characterized as loops, remains incomplete without addressing the role of intervening chromatin. Here, we examine the topology of the entire *Sonic hedgehog* (*Shh*) regulatory domain in individual alleles from the mouse embryonic forebrain. Through sequential Oligopaint labeling and super-resolution microscopy, we find that the *Shh* locus maintains a compact structure that adopts several diverse configurations independent of *Shh* expression. The most frequent configuration contained distal E-P contacts at the expense of those more proximal to *Shh*, consistent with an interconnected loop. Genetic perturbations demonstrate that this long-range E-P communication operates by *Shh*-expression-independent and dependent mechanisms, involving CTCF binding sites and active enhancers, respectively. We propose a model whereby gene regulatory elements secure long-range E-P interactions amid an inherent architectural framework to coordinate spatiotemporal patterns of gene expression.

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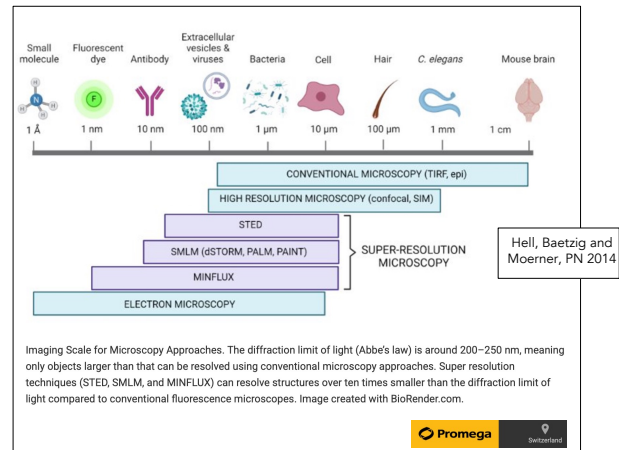
43



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*STORM: Stochastic Optical Reconstruction Microscopy



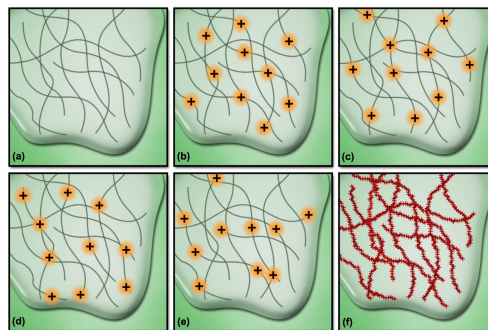
45



*STORM: Stochastic Optical Reconstruction Microscopy

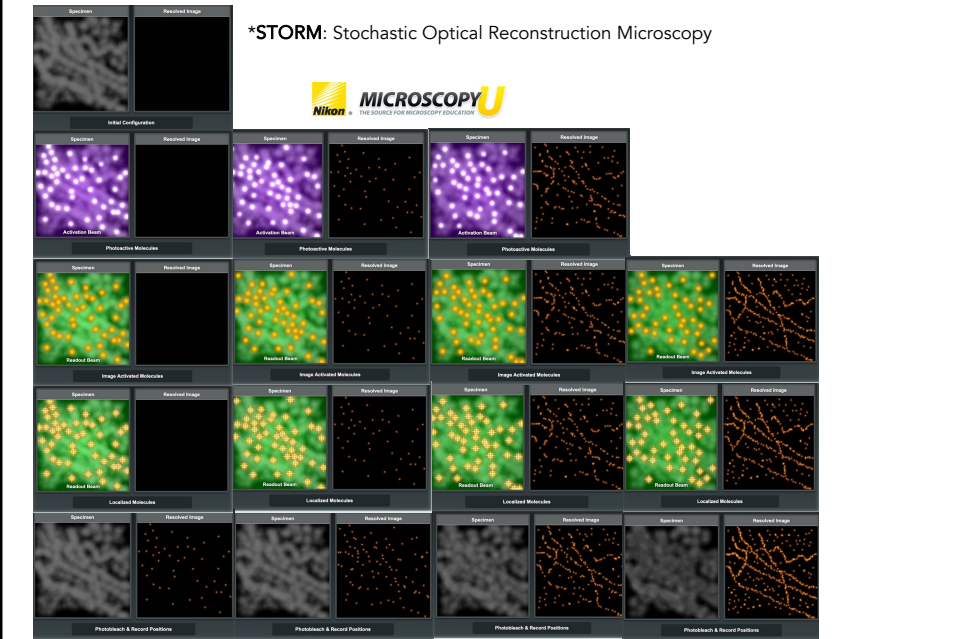
Figure 1 - Basic Principles of STORM Superresolution Imaging

*Rétération d'activation (activation beam) de la fluorescence, suivie du photoblanchiment spontané (photobleaching). Les photons émis sont capturés par un read-out beam et une image est reconstruite, qui est affinée et complétée après chaque 'cycle'....



After capturing the images with a digital camera, the point-spread functions of the individual molecules are localized with high precision based on the photon output before the probes spontaneously photobleach or switch to a dark state. The positions of localized molecular centers are indicated with black crosses. The process is repeated in Figures 1(c) through 1(e) until all of the fluorescent probes are exhausted due to photobleaching or because the background fluorescence becomes too high. The final superresolution image (Figure 1(f)) is constructed by plotting the measured positions of the fluorescent probes.

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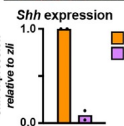
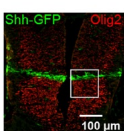
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Plan et orientation de la section histologique

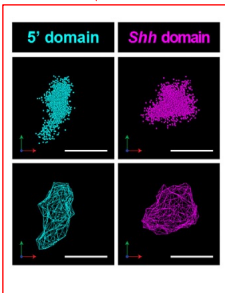


La Zli est marquée par une *Shh*-GFP (vert) et le thalamus par *Olig2* (magenta)

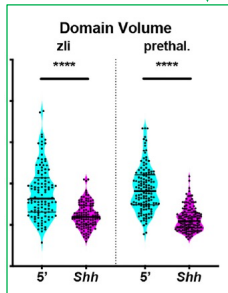


Mesures le volume de chaque domaine

Exprimé ou pas, le *Shh* domaine est plus compact



To determine if the 3D morphology of the *Shh* TAD differed with *Shh* expression, we generated Oligopaint probes that span the entire *Shh* domain and another that labels the neighboring 5' domain^{55–57}. We then performed DNA-FISH on coronal sections through the mouse forebrain at E12.5, coinciding with peak *Shh* expression in the zli. *Olig2* immunostaining was used to distinguish the *Shh*-expressing zli (*Olig2*⁺) from surrounding forebrain regions (*Olig2*⁻) that do not express *Shh* (Figures 1D and 1E). We used stochastic optical reconstructive microscopy (STORM)⁵⁸ to generate single-molecule super-resolution images and measured the precise volumes of each domain, normalizing for their corresponding genomic content (Figures 1F, 1G, S1B, and S1C). The *Shh* domain was significantly more compact and exhibited less variance than the neighboring 5' domain in both the *Shh*-expressing zli and non-expressing prethalamus, 4.25-fold and 3.4-fold, respectively (*****p* < 0.0001, Figures 1G and S1C; Table S6B). These observations suggest that the *Shh* locus maintains a relatively compact and restricted topology independent of *Shh* expression.



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Résolution des sous-structures possibles du locus

30-kb bins/
Order of hyb

Images:

Align 3-D Configuration Distances (nm)
Contact Frequency (distance threshold)

Analyse séquentielle en FISH et super-résolution en testant séparément des 'bins' de 30kb (couleurs..). Ils regardent les espaces occupés par les bins les unes après les autres...(dans les cellules de la Zli)

La bin contenant le gène *Shh* co-occupe un espace avec deux bins distales, reflétant des interactions potentielles

Ensemble Contact Frequency
($n_{\text{img}} = 1,109$)

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Résolution des sous-structures possibles du locus

*Les contacts entre SBE1/*Shh* (même bin) et SBE5 et la ZRS sont clairement significatifs .

*Les sites CTCF sont probablement impliqués dans ces contacts distaux...

Contacts with the *Shh* bin

Contact Frequency (< 200 nm)

bins 1 8 16 24 32

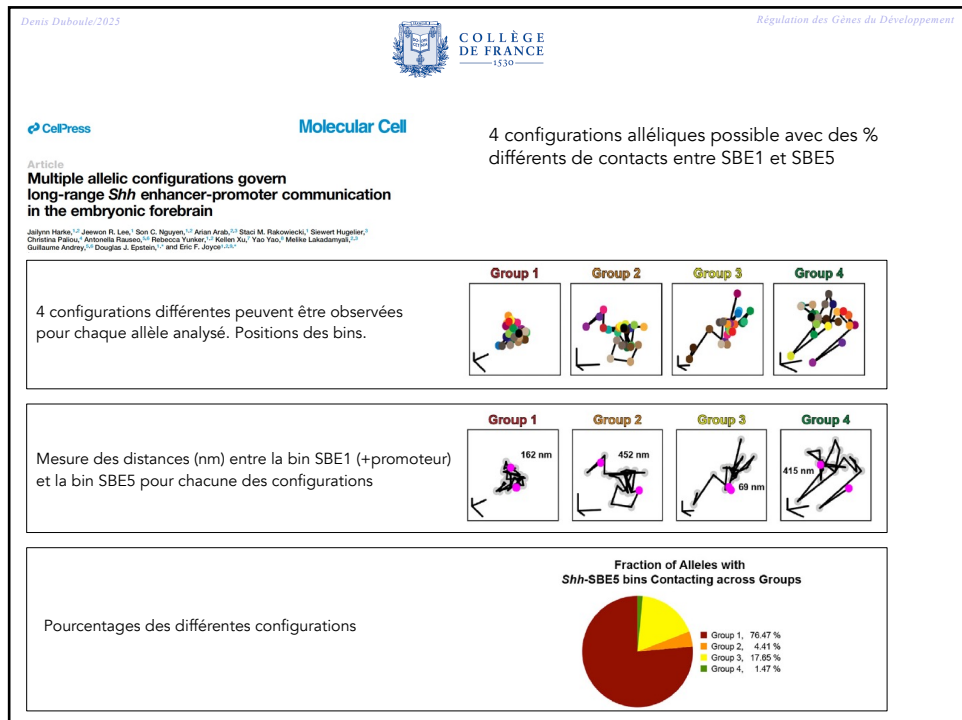
SBEs/ZRS

CTCF

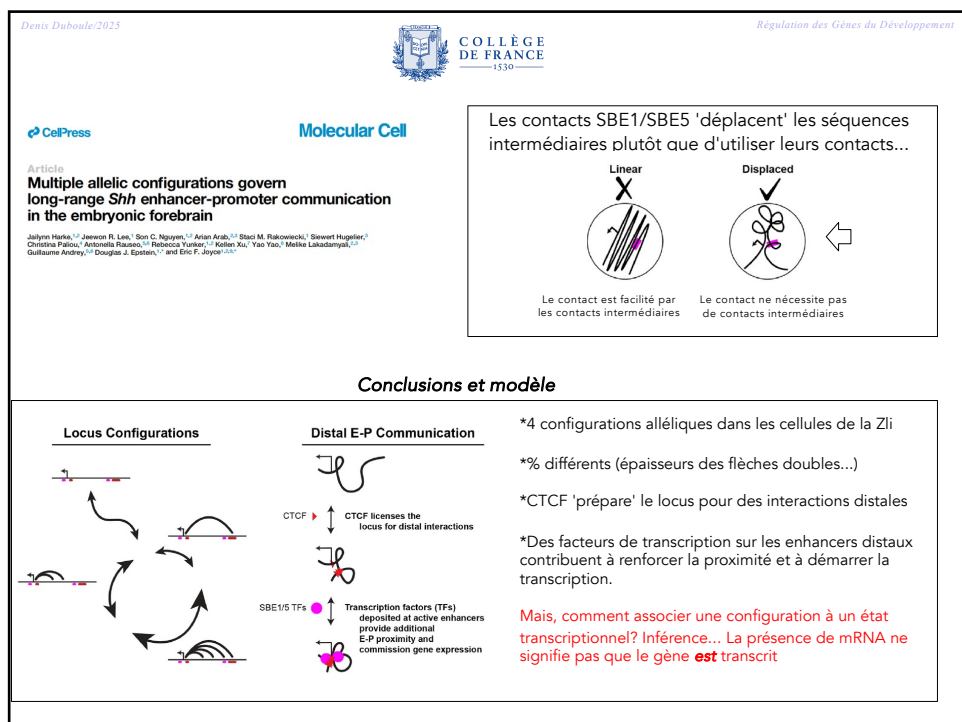
SBE1 SBE5 ZRS

*En se focalisant sur les contacts entre SBE1/*Shh* et SBE5, les auteurs notent la présence de 4 configurations alléliques différentes possibles.

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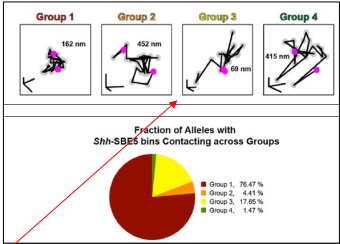
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Group	Fraction (%)
Group 1	76.47 %
Group 2	4.41 %
Group 3	17.85 %
Group 4	1.67 %

Conclusions et modèle

The looping model of E-P communication is predicated on an abundance of data identifying high-frequency contacts or physical proximity between pairs of loci and oversimplifies the configuration of the intervening chromatin. Many techniques bolstering the looping model fail to capture simultaneous, multiway interactions of individual alleles in expressing tissues. Using sequential DNA-FISH, we discovered diverse configurations that underlie a compact *Shh* locus (Figures 2 and 3). We found that only 8% of the allelic configurations resembled a classic loop structure, and of those, only 10% had a singular, exclusive contact with the bin containing *Shh*. Instead, the vast majority (77%) of alleles exhibited diverse multiway contacts along the entire length of the locus. Even within the most compact allelic configuration, we observed a toggling of distal E-P interactions at the expense of more proximal chromatin, an observation that may reconcile some conflicting models relating de-compaction to E-P activity at the *Shh* locus.^{8,34} These allelic configurations challenge the simple looping model by revealing a compact topology with multiple connections.

- *4 configurations alléliques dans les cellules de la Zli
- *% différents (épaisseurs des flèches doubles...)
- *CTCF 'prépare' le locus pour des interactions distales
- *Des facteurs de transcription sur les enhancers distaux contribuent à renforcer la proximité et à démarrer la transcription.

Mais, comment associer une configuration à un état transcriptionnel? Inférence... La présence de mRNA ne signifie pas que le gène **est** transcrit à ce moment là.