绵羊*FecB*基因编码蛋白的序列、结构和功能分析

Sequence, structure and function analysis of the protein encoded by the Sheep FecB gene

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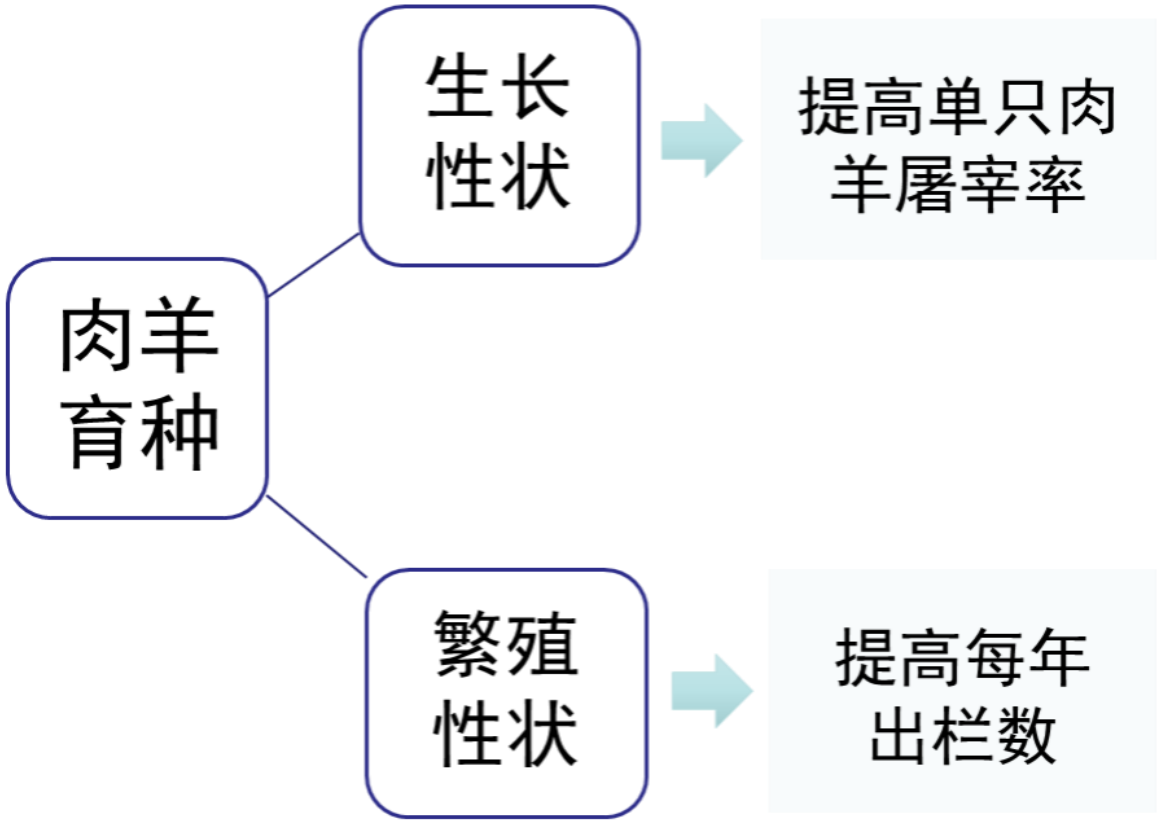


绵羊的繁殖性状是绵羊的重要经济性状，为数量性状，属低遗传力性状。它包括受胎率、多胎性（产羔率及每胎产羔数）及羔羊存活率 3 个方面，其中多胎性是决定养羊业效益的最主要的性状之一，也是绵羊业多产高产的基础。

一. 研究背景：绵羊多羔主效基因*FecB*的发现

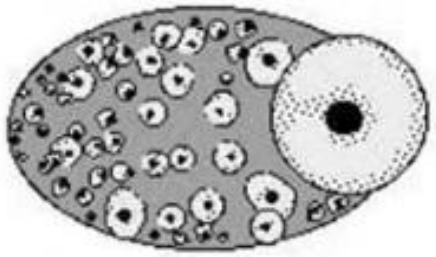
1919 年，澳大利亚 B.Seear 在新南威尔士州中毛型美利奴羊中选出一只高繁殖力母羊，并把它的后代作为一个分离群，他的侄儿 J. Seear 和 D.Seear 兄弟继续对高繁殖力性状进行选择，并以其牧场的名字命名为 Booroola 美利奴羊。

1958 年，澳大利亚联邦科学与工业研究组织用 Seears 兄弟 Booroola 羊群 1 只公羊和 13 只一胎产三羔和一胎产四羔的母羊继续扩繁而成为今天的 Booroola 绵羊。

1980 年，澳大利亚和新西兰进行的研究证实了 Booroola 绵羊高繁殖力属单基因遗传，通常认为这一遗传突变是由点突变、重复或缺失引起的，之后将该主效基因命名为 F。

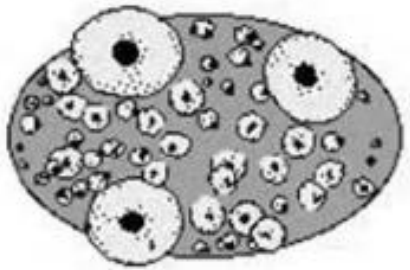
1989 年，该基因被国际绵羊和山羊遗传命名委员会命名为 *FecB*（既 Fec=fecundity，B=Booroola）

一. 研究背景：*FecB*对表型影响



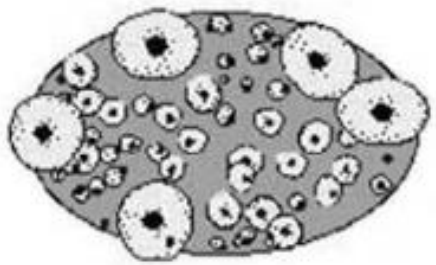
Wild type +/+

Ovulation rate = 1
Size of ovulatory follicles 7mm



Heterozygous +/B

Ovulation rate = 3
Size of ovulatory follicles 4.5mm



Homozygous B/B

Ovulation rate = 5
Size of ovulatory follicles 3.5mm

依靠后裔测定数据和排卵数、产羔数的记录数据，有报道指出*FecB*基因对排卵数和产羔数性状的影响。

一. 研究背景：*FecB*基因定位

2001 年 Wilson 等、Souza 等和 Mulsant 等几乎同时发现， *FecB* 基因所在的绵羊 6号染色体区域同线性的人 4 号染色体 q22～q23 上的骨形态发生蛋白受体 IB 基因(bone morphogenetic protein receptor-IB gene，*BMPR-IB*)，与 Booroola Merino 羊的排卵率有关联。

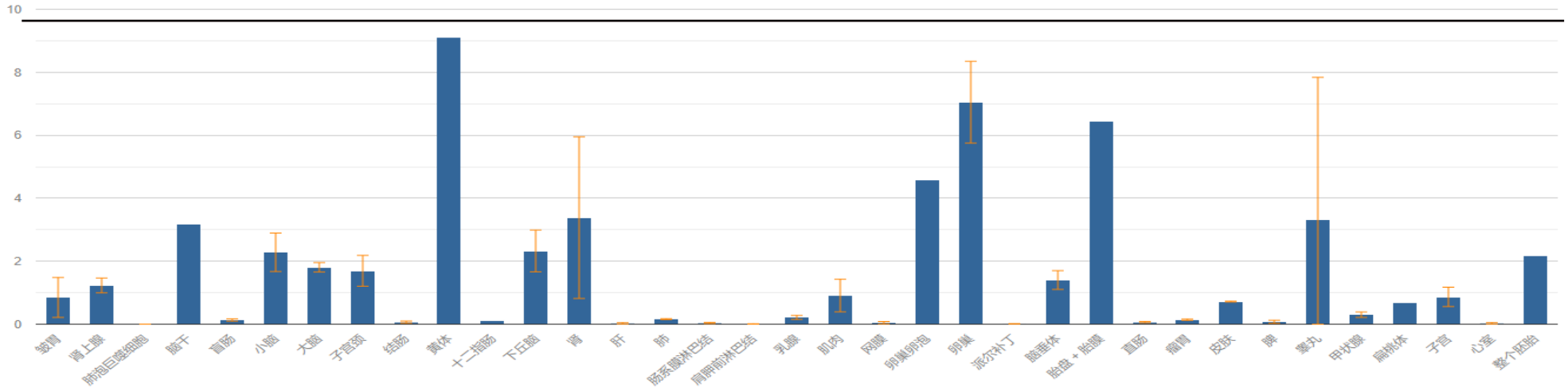
野生型（+/+）	杂合型（+/B）	纯合型（B/B）
Q249 Q249	Q249 Q249R	Q249R Q249R

*BMPR-IB*基因的A746G位置的突变,导致249位置处的谷氨酰胺转变为精氨酸

二. 基础信息：基因(NCBI)、蛋白(UniProt)

Gene ID	Description	Gene type	Location	Exon count	CDS
443454	bone morphogenetic protein receptor type 1B	protein coding	chromosome 6	17	1509bp

UniProt ID	Protein	Superfamily	Family	Subfamily	Length
A0A6P3CWH2	Serine/threonine-protein kinase receptor	Protein kinase superfamily	TKL Ser/Thr protein kinase family	TGFB receptor subfamily	502aa



Sheep Tissue Atlas (Sheep total RNA was extracted from embryonic and adult tissues)

三. 序列比对：双序列比对（Needle）

物种	登录号	得分	相同位点	相似位点	空位
人/绵羊	O00238/A0A6P3CWH2	2654.0	494/502(98.4%)	500/502(99.6%)	0/502(0%)

四.BLAST：PBLAST（NCBI）

RecName: Full=Bone morphogenetic protein receptor type-1B; Short=BMP type-1B receptor; Short=BMPR-1B; AltN...	Homo sapiens	1015	1015	100%	0.0	98.41%	502	O00238.1
RecName: Full=Bone morphogenetic protein receptor type-1B; Short=BMP type-1B receptor; Short=BMPR-1B; AltN...	Mus musculus	1014	1014	100%	0.0	98.61%	502	P36898.1
RecName: Full=Bone morphogenetic protein receptor type-1B; Short=BMP type-1B receptor; Short=BMPR-1B; AltN...	Gallus gallus	945	945	100%	0.0	92.23%	502	Q05438.1
RecName: Full=Bone morphogenetic protein receptor type-1A; Short=BMP type-1A receptor; Short=BMPR-1A; AltN...	Homo sapiens	743	743	97%	0.0	73.73%	532	P36894.2
RecName: Full=Bone morphogenetic protein receptor type-1A; Short=BMP type-1A receptor; Short=BMPR-1A; AltN...	Mus musculus	743	743	97%	0.0	73.93%	532	P36895.1
RecName: Full=Bone morphogenetic protein receptor type-1A; Short=BMP type-1A receptor; Short=BMPR-1A; AltN...	Rattus norvegicus	742	742	100%	0.0	72.67%	532	Q78EA7.1
RecName: Full=TGF-beta receptor type-1; Short=TGFR-1; AltName: Full=Serine/threonine-protein kinase receptor ...	Rattus norvegicus	498	498	95%	2e-172	53.93%	501	P80204.1
RecName: Full=TGF-beta receptor type-1; Short=TGFR-1; AltName: Full=ESK2; AltName: Full=Transforming growth...	Mus musculus	495	495	95%	2e-171	53.91%	503	Q64729.1
RecName: Full=TGF-beta receptor type-1; Short=TGFR-1; AltName: Full=TGF-beta type I receptor; AltName: Full=T...	Sus scrofa	490	490	95%	3e-169	53.39%	503	Q5CD18.1
RecName: Full=TGF-beta receptor type-1; Short=TGFR-1; AltName: Full=TGF-beta type I receptor; AltName: Full=T...	Bos taurus	488	488	96%	1e-168	52.36%	499	O46680.1
RecName: Full=TGF-beta receptor type-1; Short=TGFR-1; AltName: Full=Activin A receptor type II-like protein kinas...	Homo sapiens	483	483	94%	2e-166	53.61%	503	P36897.1

Family & Domains

Domains and Repeats

Feature key	Position(s)	Description
Domain	174 – 203	GS InterPro annotation
Domain	204 – 494	Protein kinase InterPro annotation

Region

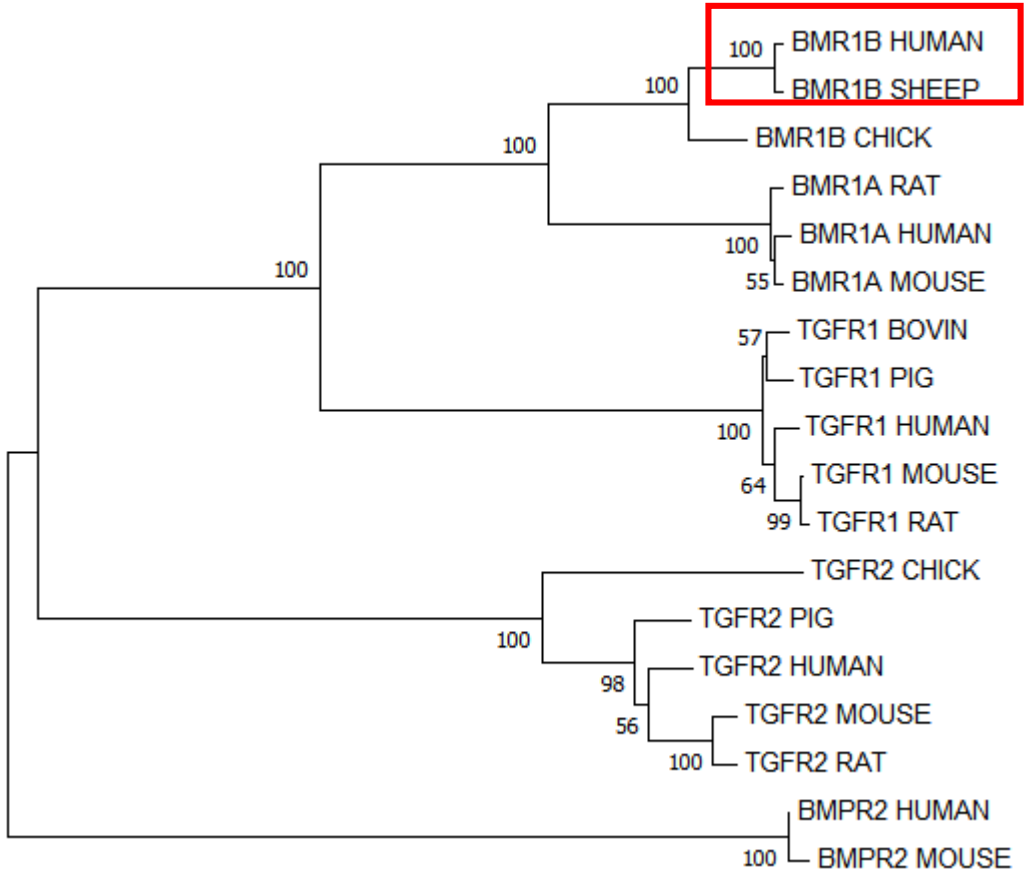
Feature key	Position(s)	Description
Region	1 – 24	Disordered Sequence analysis

Sequence similarities

Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family. TGFB receptor subfamily.

五. 系统发育树：不同物种TGF-β受体家族进化关系（MEGA7）

骨形态发生蛋白受体（BMPRs）家族作为转化生长因子受体（TGFRs）超家族中最大的一支，广泛分布在动物体内，依靠卵巢功能性的旁分泌/自分泌调节体系，对哺乳动物早期胚胎发育、卵泡发育、生殖激素调节以及卵子发生起重要调节作用。



六. 蛋白结构：Sheep→Human

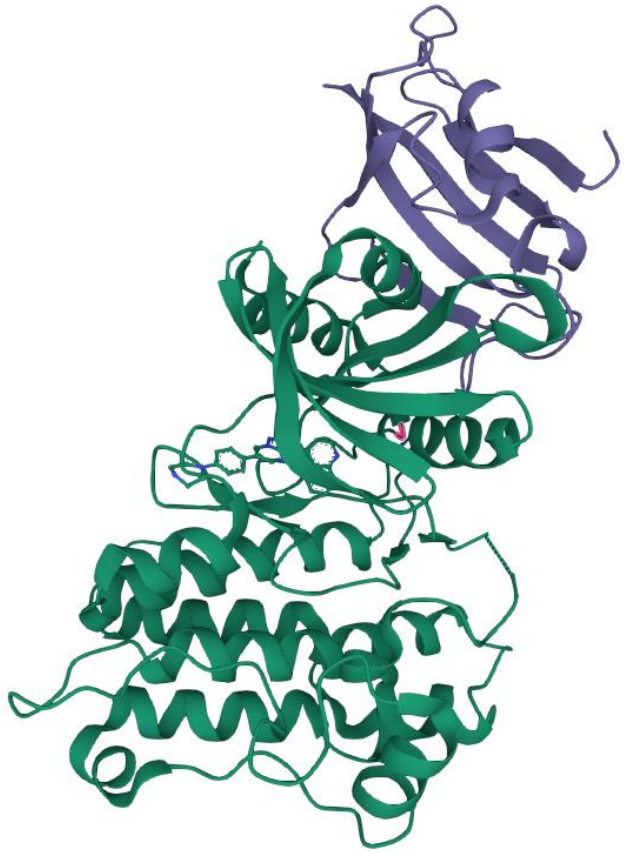
Structure[i]

Sheep

No structure information available for A0A6P3CWH2

Human

PDB	3MDY	X-ray	2.05 Å	A/C	168-502	PDBe · RCSB-PDB · PDBj · PDBsum	
AlphaFold	AF-O00238-F1	Predicted			1-502	AlphaFold	



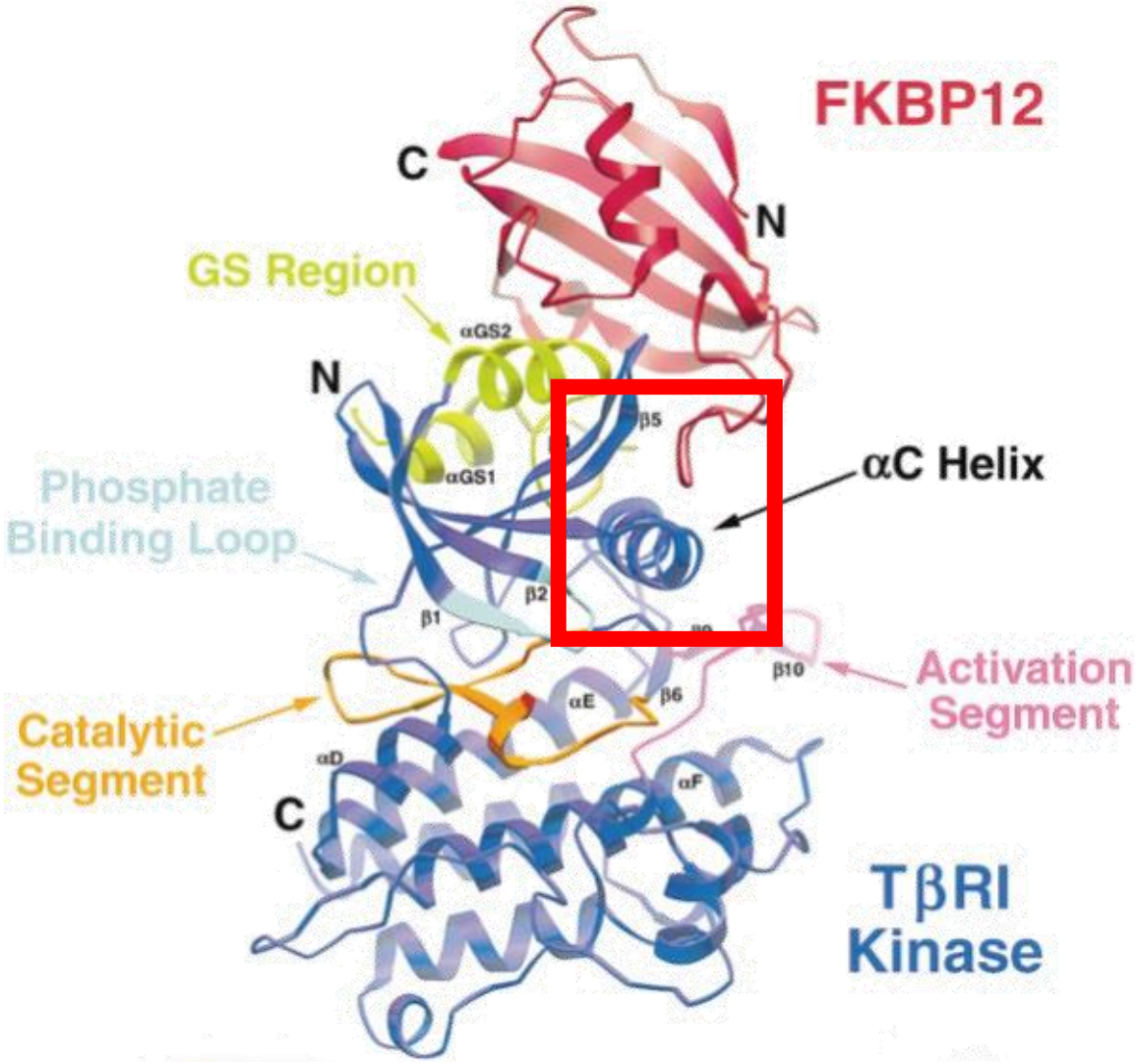
3MDY--PDB数据库

骨形态发生蛋白受体1b (BMPR1B)与FKBP12和LDN-193189复合物细胞质域的晶体结构

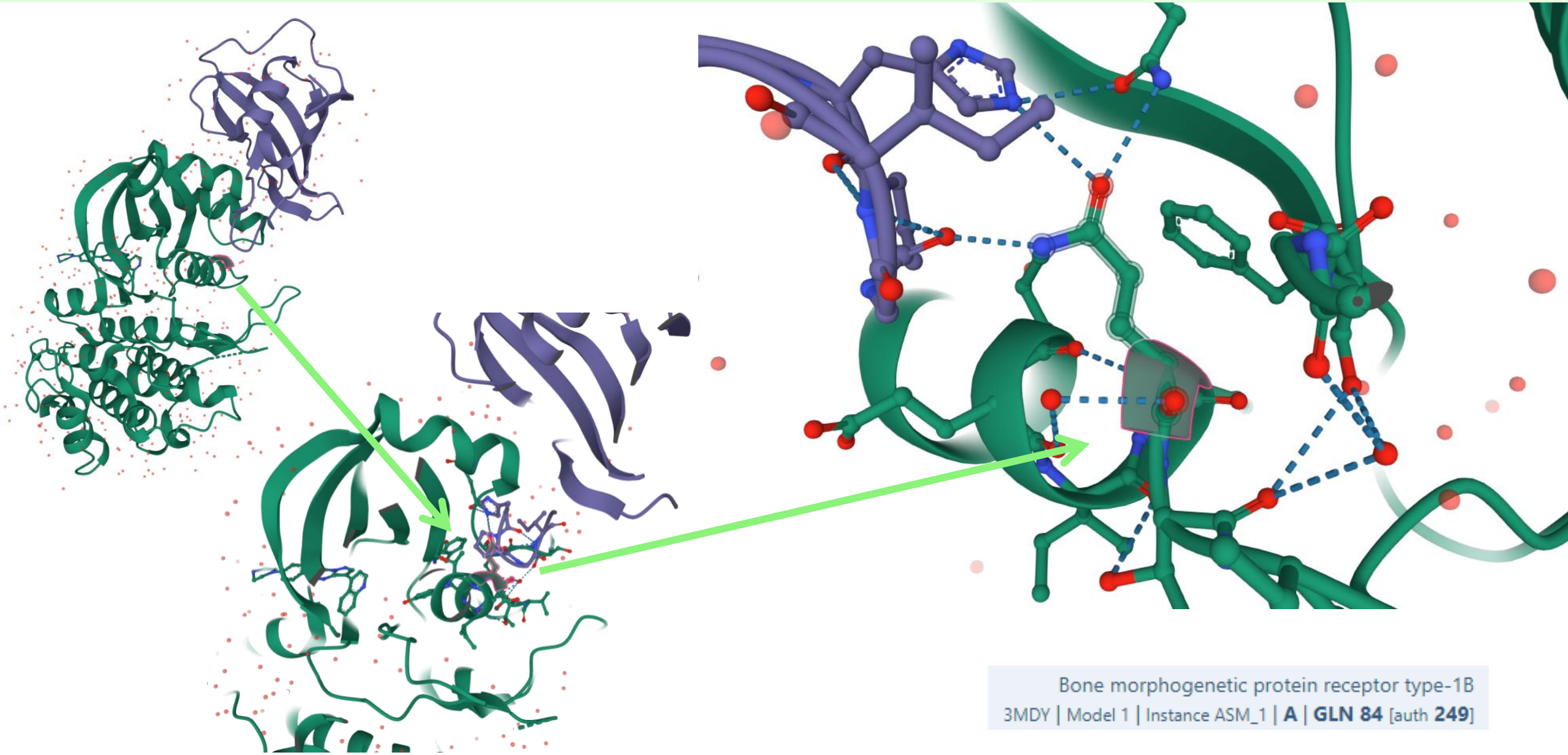
FKBP12是人TGF-β受体（TβR-I）家族成员活性的反馈调节剂，能与BMPR1B受体结合，抑制BMPR1B与配体的结合能力。

六. 蛋白结构：Human→Sheep

绵羊BMPR1B蛋白空间结构根据人TGF-β受体（TβR-I）和克莫司结合蛋白-12（FKBP12）构建成为一个复合模型。绵羊BMPR1B的FecB突变（Q249R）位于GS结构域和L45环之间，与人TGF-β受体结构域中的Q250残基相对应，Q250残基位于与FKBP12分子相连的α-C螺旋的C端。



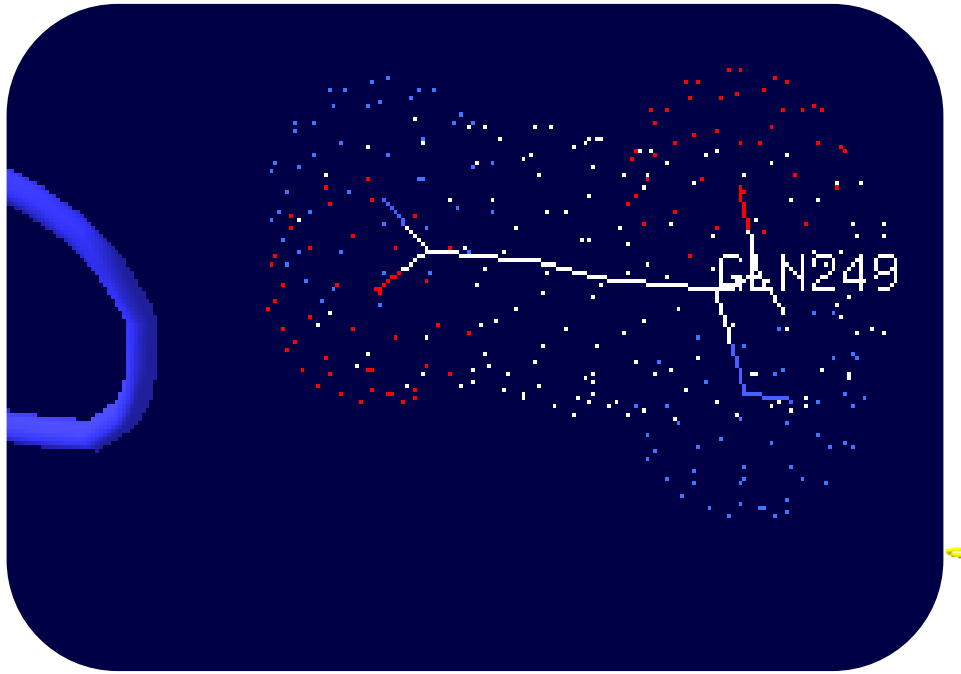
七.结构改变引起功能改变：PDB(3D-view)

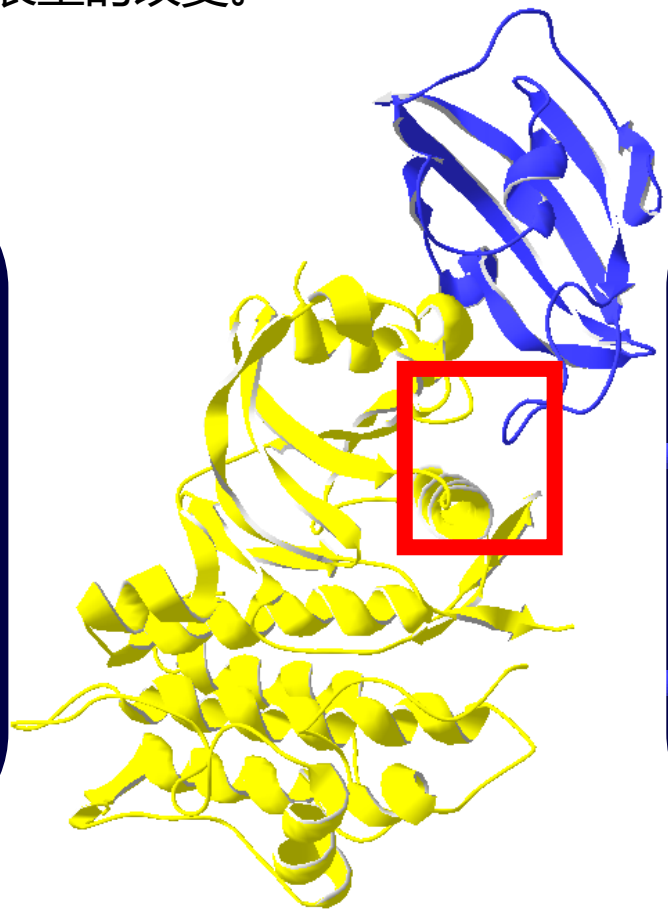


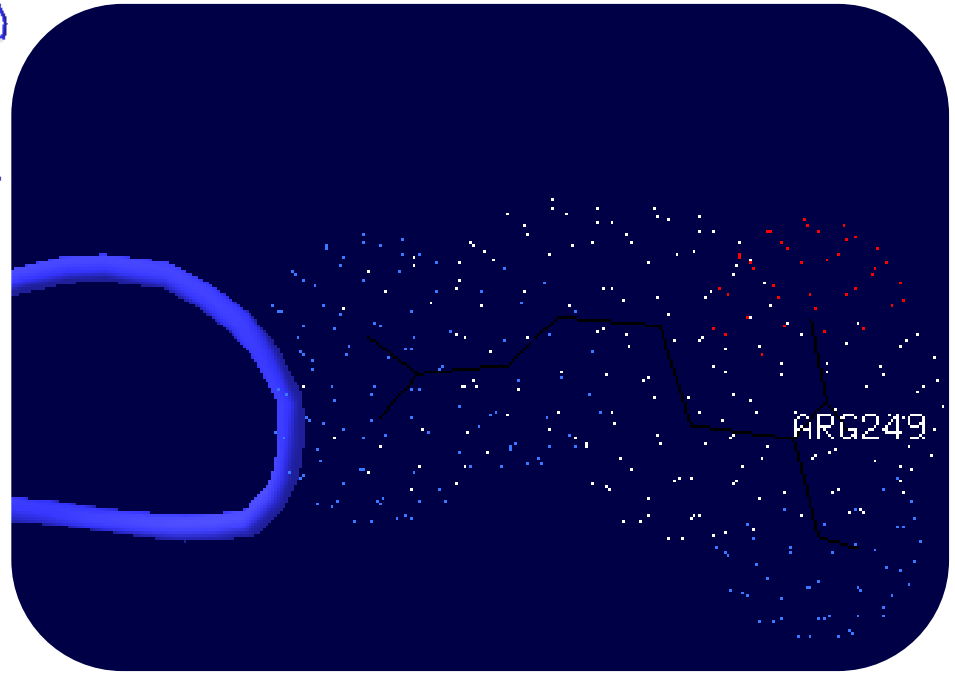
七. 结构改变引起功能改变：PdbViewer

Booroola绵羊的Q249R突变使得BMPR1B Q250与FKBP12形成氢键，增强了FKBP12与BMPR1B之间的π电子的相互作用，进而结合的更紧密，导致对BMPR1B受体活性的抑制作用增加。

所以，可以认为Q249R突变使得FKBP12对BMPR1B活性抑制作用的加强，细胞对BMPR1B特异性配体的敏感性变化，最终导致细胞活性的改变，以至于最后表型的改变。







八. 研究展望：技术落地

申请号	申请日	发明名称	发明人
201510594573.6	2015/09/17	利用Taqman MGB探针检测绵羊*FecB*基因多态性的方法	储明星，刘秋月，狄冉，胡文萍，王翔宇，王贵，潘章源，赵万民，李虎山
201510304092.7	2015/06/04	利用SNaPshot技术检测绵羊*FecB*基因多态性的方法	储明星，刘秋月，狄冉，胡文萍，王翔宇，王贵，潘章源，赵万民，李虎山

八. 研究展望：机理研究

frontiers in Cell and Developmental Biology

ORIGINAL RESEARCH
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Transcriptome Analysis Reveals Differentially Expressed Genes and Long Non-coding RNAs Associated With Fecundity in Sheep Hypothalamus With Different FecB Genotypes

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Small-tailed Han sheep, with different *FecB* genotypes, manifest distinct ovulation rates and fecundities, which are due to differences in reproductive hormones secreted by the hypothalamic–pituitary–ovarian axis. Nevertheless, the function of the hypothalamus against a *FecB* mutant background on increasing ovulation rate is rarely reported. Therefore, we determined the expression profiles of hypothalamus tissue collected from six wild-type (WW) and six *FecB* mutant homozygous (BB) ewes at the follicular and luteal phases by whole-transcriptome sequencing. We identified 53 differentially expressed mRNAs (DEGs) and 40 differentially expressed long non-coding RNAs (DELs) between the two estrus states. Functional annotation analysis revealed that one of the DEGs, PRL, was particularly enriched in the hypothalamic function, hormone-related, and reproductive pathways. The lncRNA–target gene interaction networks and KEGG analysis in combination suggest that the lncRNAs LINC-676 and WNT3-AS *cis*-acting on *DRD2* and *WNT9B* in different phases may induce gonadotropin-releasing hormone (GnRH) secretion. Furthermore, there were differences of regulatory elements and WNT gene family members involved in the follicular–luteal transition in the reproductive process between wild-type (*WNT7A*) and *FecB* mutant sheep (*WNT9B*). We combined the DEG and DEL data sets screened from different estrus states and genotypes. The overlap of these two sets was identified to select the mRNAs and lncRNAs that have major effects on ovulation. Among the overlapping molecules, seven DEGs and four DELs were involved in the follicular–luteal transition regulated by *FecB* mutation. Functional annotation analysis showed that two DEGs (*FKBP5* and *KITLG*) were enriched in melanogenesis, oxytocin, and GnRH secretion. LINC-219386 and IGF2-

RESEARCH ARTICLE

Hypothalamic Proteome

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Identification of Prolificacy-Related Differentially Expressed Proteins from Sheep (*Ovis aries*) Hypothalamus by Comparative Proteomics

Zhuangbiao Zhang, Jishun Tang, Ran Di, Qiuyue Liu, Xiangyu Wang, Shangquan Gan, Xiaosheng Zhang, Jinlong Zhang, Wei Chen, Wenping Hu, and Mingxing Chu**

Reproduction, as a physiologically complex process, can significantly affect the development of the sheep industry. However, a lack of overall understanding to sheep fecundity has long blocked the progress in sheep breeding and husbandry. In the present study, the aim is to identify differentially expressed proteins (DEPs) from hypothalamus in sheep without FecB mutation in two comparison groups: polytocous (PF) versus monotocous (MF) sheep at follicular phase and polytocous (PL) versus monotocous (ML) sheep at luteal phase. Totally 5058 proteins are identified in sheep hypothalamus, where 22 in PF versus MF, and 39 proteins in PL versus ML are differentially expressed, respectively. A functional analysis is then conducted including Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis to reveal the potential roles of these DEPs. The proteins ENSOARP00000020097, ENSOARP00000006714, growth hormone (GH), histone deacetylase 4 (HDAC4), and 5′-3′ exoribonuclease 2 (XRN2) in PF versus MF, and bcl-2-associated athanogene 4 (BAG4), insulin-like growth factor-1 receptor (IGF1R), hydroxysteroid 11-beta dehydrogenase 1 (HSD11B1), and transthyretin (TTR) in PL versus ML appear to modulate reproduction, presumably by influencing the activities of gonadotropin-releasing hormone (GnRH). This study provides an alternative method to identify DEPs associated with sheep prolificacy from the hypothalamus. The mass spectrometry data are available via ProteomeXchange with identifier PXD013822.

1. Introduction

The achievement of reproduction is an essential but complex process involving the central nervous system and endocrine activities, especially those involving hormones released from the hypothalamic-pituitary-gonadal axis.[1] The hypothalamus, as a critical organ in this axis, initiates the reproductive process through a gonadotropin-releasing hormone (GnRH) signal, which then travels to the pituitary, where follicle-stimulating hormone and luteinizing hormone are induced to function in both the ovary and testis, enabling the synthesis and release of ovarian estradiol and testicular hormone, facilitating folliculogenesis and spermatogenesis, respectively, and maintaining the functions of corpus luteum.[2,3] Numerous studies involving GnRH and several neuroendocrine factors, such as kisspeptins and neurokinin B, known to be related to GnRH release[4–6] have been widely studied. Furthermore, some metabolic

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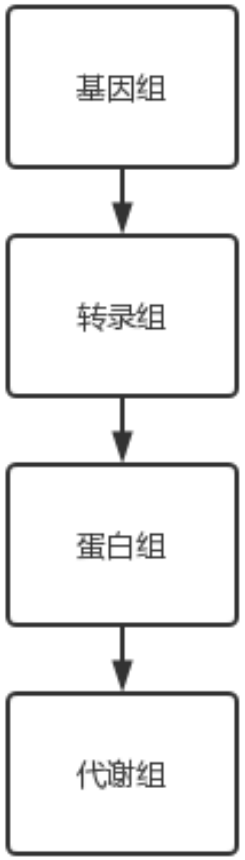

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T H A N K S