Evolution and gene regulation of the genomic imprinting mechanism

by

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Abstract

Genomic imprinting describes an epigenetic mechanism by which genes are active or silent depending on their parental origin. Imprinting exists in plants and mammals, but how this monoallelic expression mechanism has evolved is not understood at the molecular level. Here I describe the mapping, sequencing and analysis of vertebrate orthologous imprinted regions spanning 11.5 Mb of genomic sequence from species with and without genomic imprinting. In eutherian (placental) mammals, imprinting can be regulated by differential DNA methylation, non-coding RNAs, enhancers and insulator elements. The systematic sequence comparison of the *IGF2-H19* imprinting cluster, in eutherians and marsupials (tammar wallaby and opossum), has revealed the presence of the enigmatic non-coding RNA *H19* in marsupials. Furthermore, we have characterised the marsupial *H19* expression status and identified key regulatory elements required for the germline imprinting of the neighbouring *IGF2* gene. All the major hallmarks of the imprinting mechanism of the *IGF2-H19* locus were found to be conserved in therian mammals. In mammals, this imprinting system is therefore the most conserved germline derived epigenetic mechanism discovered so far.

The high-quality genomic sequences have provided early glimpses of the genomic landscapes for species such as the monotreme platypus and marsupial tammar wallaby for which little was previously known. Comparative sequence analysis was used to identify candidate regulatory elements in the neighbouring imprinting centre 1 and 2 regions of human chromosome 11p15.5. Nine novel enhancer elements were identified following *in vitro* gene-reporter assays and correlation of conserved sequences with recent ENCODE data revealed probable functions for a further 24 elements.

This project has led to the formation of the Sequence Analysis of Vertebrate Orthologous Imprinted Regions (SAVOIR) consortium and resources developed here are being used by the imprinting community to further our knowledge of the evolution of the genomic imprinting mechanism.

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Abbreviations used in this thesis

Abbreviation	Description
3C	Capturing Chromosome Conformation
aa	Amino Acid
ACeDB	A C. elegans DataBase
AGI	Arizona Genomics Institute
AGP	A Golden Path
AGRF	Australian Genome Research Facility
BAC	Bacterial Artificial Chromosome
BCM-HGSC	Baylor College of Medicine – Human Genome Sequencing Center
BLAST	Basic Local Alignment Search Tool
BLAT	Basic Local Alignment Tool
bp	Base pair(s)
BSA	Bovine Serum Albumin
BWS	Beckwith-Wiedemann Syndrome
C+G	Cytosine and guanine content
cDNA	Complementary DNA
CDS	CoDing Sequence
CFTR	Cystic fibrosis transmembrane conductance regulator
CTCF	CCCTC binding factor
CUGI	Clemson University Genomics Institute
DMD	Differentially methylated domain (5' of H19 gene)
DMR	Differentially methylated region
DNA	DeoxyriboNucleic Acid
EBI	European Bioinformatics Institute
ECR	Evolutionary Conserved Region
EMBL	European Molecular Biology Laboratoriums
EMBOSS	European Molecular Biology Open Software Suite

ENCODE	ENCyclopedia Of DNA Elements
ePCR	Electronic PCR
ERV	Endogenous Retrorvirus
EST	Expressed Sequence Tag
FASTA	DNA and protein file format
FISH	Fluorescent In Situ Hybridisation
FPC	FingerPrinting Contigs
Gb	Giga-basepairs
H3K9	Histone 3 lysine 9
H3K27	Histone 3 lysine 27
HAVANA	Human And Vertebrate Analysis aNd Annotation
HGP	Human Genome Project
HTML	Hypertext Markup Language
HUGO	Human Genome Organisation
IC	Imprinting Centre
Imprintace	Imprinting implementation of ACeDB
kb	kilo base pairs
kg	kilogram
LCR	Locus Control Region
LINE	Long Interspersed Nuclear Element
LTR	Long Terminal Repeat
MAR	Matrix Attachment Region
Mb	Megabase pairs
mg	Milligram
MIR	Mammalian-wide Interspersed Repeat
mm	Millimetre
MGSC	Mouse Genome Sequencing Consortium
MIR	Mammalian-wide Interspersed Repeat
MMU7	Mus Musculus chromosome 7
mRNA	Messenger RNA
miRNA	Micro RNA

Myr	Million years
NCBI	National Center for Biotechnology Information
ncRNA	Non-coding RNA
NIH	National Institutes of Health
nt	Nucleotide
OMIM	Online Mendelian Inheritance of Man
ORF	Open Reading Frame
PAC	P1-derived Artificial Chromosome
PCR	Polymerase Chain Reaction
pg	Picogram
PIP	Percentage Identity Plot
PSV	Paralogous Sequence Variants
PWS/AS	Prader-Willi/Angelman Syndrome
QH	Quantitative Hypervariable
QTL	Quantitative Trait Loci
RACE	Rapid Amplification of cDNA Ends
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
RT-PCR	Reverse Transcription PCR
SAVOIR	Sequence Analysis of Vertebrate Orthologous Imprinted Regions
SCF	Standard Chromatograph Format
SINE	Short Interspersed Nuclear Element
snoRNA	Small nucleolar RNA
SNP	Single Nucleotide Polymorphism
SNV	Single Nucleotide Variants
STS	Sequence Tagged Site
TF	Transcription Factor
TFBS	Transcription Factor Binding Site
TPF	Tile Path Format
TRF	Tandem Repeat Finder

tRNA	Transfer RNA
TSS	Transcription Start Site
UCSC	University of California, Santa Cruz
UMIST	University of Manchester Institute of Science and Technology
UPD	Uniparental disomies
pUPD	Paternal UPD
UTR	UnTranslated Region
VEGA	Vertebrate Genome Annotation
VISTA	Program for visualising global DNA sequence alignments
rVISTA	Regulatory VISTA
WGS	Whole Genome Shotgun
WUGSC	Washington University Genome Sequencing Center
WWW	World Wide Web
XCI	X chromosome inactivation

Publications arising from this work

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