Investigating the evolution of replication timing and monoallelic expression in mammals and birds

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Abbreviations

°C	Degree celsius
µg	Microgram
µl	Microlitre
µm	Micrometre
3C	Chromosome conformation capture
4C	Chromosome conformation capture-on-chip
5C	Chromosome conformation capture carbon copy
ACT	Associated chromosome trap
AER	Allele expression ratio
BrdU	Bromodeoxyuridine
cDNA-seq	cDNA library sequencing
ChIA-PET	Chromatin Interaction Analysis by Paired-End Tag sequencing
CT	Chromosome territory
CTCF	CCCTC-binding factor
DD	Double-double dot DNA FISH signal (replicated locus)
DMR	Differentially methylated region
EIO	Eutherian imprinted ortholog
EIPO	Eutherian imprinted platypus ortholog
FISH	Fluorescent *in situ* hybridisation
G1	Gap 1
Hi-C	Genome-wide 3C experiment
Hox	Homeobox
ICR	Imprinting control regions
LCR	Locus control region
LINE	Interspersed nuclear element
MB	Mega base
MCM	Minichromosome maintenance
MHM	Male hypermethylated
ncRNA	Non-coding RNA
ND	No data avaliable
NK	Natural Killer-cell

OR	Origin of replication
ORC	Origin recognition complex
ORc	Olfactory receptor
Pre-RC	Pre-replicative complex
RNA-seq	RNA library sequencing
Rsx	RNA-on-the-silent X
RT-PCR	Reverse-transcriptase polymerase chain reaction
S-phase	DNA synthesis phase of the cell cycle
SD	Single-double dot DNA FISH signal (replicating locus)
SINE	Short interspersed nuclear element
SNP	Single nucleotide polymorphism
SS	Single-single dot DNA FISH signal (unreplicated locus)
TE	Transposable element
XIC	X-chromosome inactivation centre
Xist	X inactive specific transcript

Nomenclature

Throughout this thesis, various forms of conventional notations are observed which pertain to species-specific nomenclature, particularly mouse, human, platypus, and chicken.

Abstract

Monoallelic expression and replication timing are closely linked fundamental aspects of genome biology, yet their evolutionary trajectory has not been investigated in much detail. The monoallelic expression status of imprinted genes observed in therian species has previously not been found in the earlier-diverged monotreme mammals, or in birds, when measured using molecular techniques. Furthermore, the observation that eutherian imprinted and X-borne genes asynchronously replicate was traditionally thought to be linked to the dissimilar epigenetic states that existed at each allele controlling monoallelic expression. In this study, we use a combination of cytogenetic and molecular techniques to assess the replication status of sex chromosome genes in the platypus and chicken, as well as the replication status and expression pattern of platypus imprinted orthologs.

We find that asynchronous replication does occur at specific sex chromosome loci in platypus and chicken, although in chicken the amount of asynchronous replication changes over development. Furthermore, differential chromatin compaction is observed in platypus sex chromosomes, a characteristic observed in therian X-inactivation, suggesting that both asynchronous replication and chromatin compaction are features characteristic of amniote sex chromosomes. Asynchronous replication and monoallelic expression is observed at platypus imprinted orthologs, indicating that a ‘pre-imprinted’ status is observed at these genes in non-therian amniote species. These results show that monoallelic expression predates imprinting at these loci, suggesting that the partial monoallelic expression observed in monotreme mammals has evolved in therian mammals to become parentally-inherited imprinted expression.