Coordination of the random asynchronous replication of autosomal loci

Published online 10 February 2003; doi:10.1038/ng1102

Random monoallelic expression and asynchronous replication define an unusual class of autosomal mammalian genes. We show that every cell has randomly chosen either the maternal or paternal copy of each given autosome pair, such that alleles of these genes scattered across the chosen chromosome replicate earlier than the alleles on the homologous chromosome. Thus, chromosome-pair non-equivalence, rather than being limited to X-chromosome inactivation, is a fundamental property of mouse chromosomes.

Monoallelically expressed genes fall into three distinct classes. X inactivation in female cells is a random process resulting in half of the cells choosing the maternal X chromosome and half choosing the paternal X chromosome[1]. By contrast, autosomal imprinted genes such as *Igf2* and *H19* are monoallelically expressed according to the parent of origin[2]. The third class, randomly monoallelically transcribed autosomal genes, includes the large family of odorant-receptor genes[3] as well as genes encoding the immunoglobulins[4], T-cell receptors[5], interleukins[6,7], natural killer–cell receptors[8] and pheromone receptors[9].

All monoallelically expressed genes share the property of asynchronous replication[3,7,10], defined as one allele replicating earlier in S phase than the other allele. For most other genes, both alleles replicate synchronously at a defined portion of S phase. Asynchronous replication is an epigenetic mark that appears before transcription and may underlie the differential behavior of two alleles of identical sequence[10]. For those genes whose transcription is randomly monoallelic, the asynchronous replication is also random. The asynchronous replication seems to be established early in development before tissue-specific transcription is established[10,11] and is therefore found even in tissues in which the genes are not expressed[3]. For example, the asynchronous replication of odorant-receptor genes has been observed in all cell types analyzed, including fibroblasts and lymphocytes. The presence of asynchronous replication in a variety of cell types allowed us to compare the replication timing of diverse monoallelically expressed genes that are expressed in different cells. Given that these genes are widely dispersed across autosomes[12,13], we sought to establish the extent to which their replication asynchrony is coordinated. We focused on four autosome pairs, each containing distinct loci of randomly monoallelically expressed genes (Fig. 1*a*).

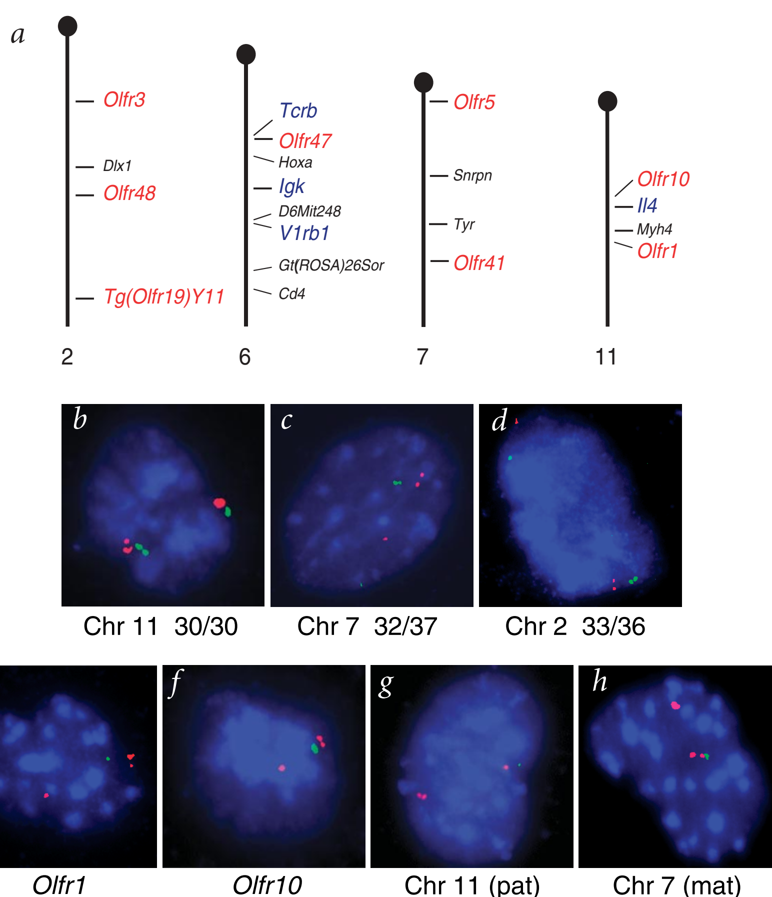

Fig. 1 Coordination of odorant-receptor asynchronous replication for individual mouse chromosome pairs. ***a***, Diagram showing the relative positions of odorant-receptor genes (red) and other monoallelically expressed genes analyzed in this study (blue) along with the location of control genes (black). Centromeric ends are at the top. ***b*–*d***, Two-color FISH analysis was done on a population of mouse embryonic fibroblasts. Blue represents DAPI staining of chromatin. ***b***, Analysis of Chromosome 11. The Cy3-labeled probe (red) identifies the *Olfr1* odorant-receptor gene and the FITC-labeled probe (green) identifies *Olfr10*. The double-dot signals for the two probes in these images are on the same chromosome, indicating coordination of these two distant loci (30 of 30 cells counted). A control probe, *Myh4*, located between the *Olfr1*and *Olfr10* loci, is synchronously replicating (9% single dot–double dot pattern). *c*, Similar analysis of Chromosome 7 for two odorant-receptor genes from distinct clusters, *Olfr5* (red) and *Olfr41* (green), showing coordination (32 of 37 cells counted). Control probes between the *Olfr5* and *Olfr41* loci included the gene encoding tyrosinase, which is synchronously replicated in wild-type cells (13% single dot–double dot pattern), and the asynchronous but imprinted gene *Snrpn*. As expected, *Snrpn* did not show coordination with an odorant receptor (data not shown). *d*, Similar analysis showing that two odorant-receptor genes from distinct clusters of Chromosome 2 were coordinated (33 of 36 cells counted): *Olfr48* (red), *Olfr3* (green). A control probe, *Dlx1*, is synchronously replicating (10% single dot–double dot pattern). ***e*,*f***, FISH analyses of line A.5 (see Supplementary Table 1 online) detected with a β-geo probe (green) that identifies the maternal Chromosome 11. Examples of cells probed with *Olfr1* (red, *e*) and *Olfr10* (red, *f*) are shown. Both of these odorant-receptor genes are maternal early-replicating in line A.5 (for data on all similar cell lines, see Supplementary Table 2 online). ***g*,*h***, Lack of coordination between Chromosomes 7 and 11. In each case, the maternal chromosome is marked by the green probe. The maternal Chromosome 11 has a β-geo insertion and the paternal Chromosome 7 has a deletion at the tyrosinase locus. Line A.1 (see Supplementary Table 3 online) shows lack of coordination between paternally early-replicating *Olfr1* on Chromosome 11 (*g*) and maternally early-replicating *Olfr41* on Chromosome 7 (*h*).

© 2003 Nature Publishing Group http://www.nature.com/naturegenetics

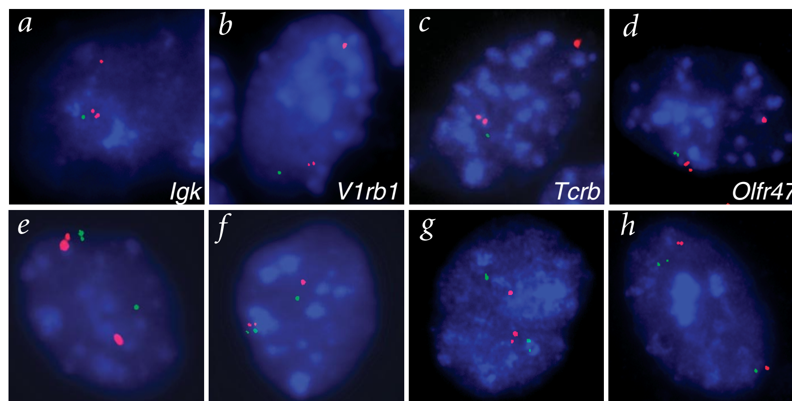

Fig. 2 A variety of monoallelically expressed genes show coordination. ***a–d***, Analyses of a clonal cell line that has a marked maternal copy of Chromosome 6 (β-geo transgene, green). Probes for the *Igk* cluster (*a*), a large *V1R* pheromone receptor cluster (VNO-61; *b*), the *Tcrb* (*c*) and an odorant-receptor cluster containing *Olfr47* (*d*) are shown (red). Line F.1 has all four genes maternally early replicating. Line C.1 (see Supplementary Fig. 2 online) has all four paternally early replicating. Two control probes, *Hoxa* and *Cd4*, each showed synchronous replication (12% and 18% single dot–double dot pattern, respectively). ***e***, Analyses of uncloned fibroblasts (similar to the analyses shown in Fig. 1*b*–*d*) also showed coordination of an odorant-receptor gene cluster on Chromosome 6 containing *Olfr47* (red) and the VNO *V1R* cluster (green; 30 of 32 cells). ***f***, Same analysis as in *e*, but comparing coordination of *Olfr47* with *Tcrb* (33 of 34 cells). ***g***, A similar population analysis showed coordination of *Il4* (red) and *Olfr10* (green) on Chromosome 11 (25 of 26 cells). ***h***, Example of coordination of endogenous *Olfr48* (green) and transgenic *Tg(Olfr19)Y11* (red) on Chromosome 2 (30 of 31 cells; $P < 0.0000001$).

© 2003 Nature Publishing Group http://www.nature.com/naturegenetics

Asynchronous replication can be assayed by fluorescence *in situ* hybridization (FISH) analysis of interphase nuclei[14]. Replicated loci are visualized as a double-dot hybridization signal, whereas unreplicated loci are visible as a single dot. Asynchronously replicating genes present a single dot–double dot pattern in 30–40% of S-phase cells, whereas synchronously replicating genes present this pattern in roughly 10–15% of S-phase cells[14]. Although the FISH assay is only an indirect assessment of replication timing, asynchronous replication observed with this assay has been corroborated by direct measurements of replication timing (refs. 10,11; see Supplementary Note 1 and Supplementary Fig. 1 online). To assess coordination of distant loci on a given chromosome, we used two-color FISH analysis to examine two genes simultaneously and scored cells that presented a single dot–double dot signal for both genes. If the two genes are coordinated, and are replicated during a overlapping portion of S phase, the double dots for both genes should be on the same chromosome (maternal or paternal) and therefore close to each other in the nucleus. If the two genes are not coordinated, the double dots for both genes should be on the same chromosome only 50% of the time.

Using this approach we assessed the potential for coordination of asynchronous replication in wild-type primary mouse embryonic fibroblasts, analyzing two distinct odorant-receptor loci on Chromosome 11 that are 14 cM apart. Notably, we observed coordination in all of the 30 cells in which both probes presented the single dot–double dot pattern (Fig. 1*b*). Similarly, we observed coordination for two distant loci on Chromosome 7 (in 32 of 37 cells) and for two distant loci on Chromosome 2 (in 33 of 36 cells; Fig. 1*c*,*d*). As expected, in each case, genes between the distinct odorant-receptor loci replicated synchronously. These data indicate that odorant-receptor genes have long-range coordination of their replication asynchrony for the three autosomes examined.

We examined whether coordinated asynchronous replication of odorant-receptor genes, once established, is heritable in the progeny of a given cell. We derived clonal cell lines from embryonic and adult mice with distinguishable maternal and paternal chromosomes for Chromosomes 7 and 11. We analyzed seven cell lines for Chromosome 7 and eight cell lines for Chromosome 11. In some cell lines we consistently observed early replication of the maternal allele (Fig. 1*e*), and in the other cell lines we consistently observed early replication of the paternal allele (see Supplementary Tables 1 and 2 online). These analyses indicate that for each odorant-receptor gene, the random choice of one of the two alleles to replicate early, once established, is heritable. Analyses of these clonal cell lines also confirmed coordination of asynchronous replication along a given chromosome. For all the cell lines analyzed, both loci replicated the same parental allele early (Fig. 1*e*,*f* and see Supplementary Tables 1 and 2 online).

To test for genome-wide coordination, we analyzed clonal cell lines derived from mice carrying marks allowing us to distinguish the parental origins of two chromosome pairs at a time. We used FISH analysis to compare the replication timing of odorant-receptor genes on Chromosomes 6 and 11 with odorant-receptor genes on Chromosome 7 in pairwise comparisons. Odorant-receptor genes on different chromosomes were not coordinated in their replicative asynchrony; we observed all possible outcomes (Fig. 1*g*,*h* and see Supplementary Table 3 online). Thus, rather than genome-wide coordination, asynchronous replication of odorant-receptor genes seems to be coordinated only at the level of each chromosome pair.

To explore whether other randomly monoallelically transcribed genes are also coordinated in their replicative asynchrony, we examined clonal cell lines in which we can distinguish the maternal and paternal copies of Chromosome 6. We observed asynchronous replication of the *Igk* constant region, a V1R pheromone-receptor gene and *Tcrb* (Fig. 2*a*–*c*). Notably, all three of these loci were coordinated with each other as well as with an odorant-receptor gene cluster on Chromosome 6 (Fig. 2*a*–*d*). In some clonal cell lines the maternal alleles of all four genes replicated early (Fig. 2*a*–*d*), and in others the paternal alleles of all four genes replicated early (see Supplementary Fig. 2 online). Analyses of uncloned populations of cells (similar to the analyses presented in Fig. 1*b*–*d*) also showed coordination of genes on Chromosome 6 (Fig. 2*e*,*f*). Similar population analyses showed that on Chromosome 11, the gene encoding interleukin-4 (*Il4*) was coordinated with the odorant-receptor genes (Fig. 2*g*). These data, taken together, indicate that all randomly asynchronously replicated genes examined are coordinated along each chromosome.

One question arising from these observations is whether the coordination mechanisms used by different chromosomes can communicate with each other if sequences from different chromosomes are artificially placed in *cis*. We analyzed a small odorant-receptor translocation that we created artificially: a 300-kb odorant receptor–containing YAC transgene derived from Chromosome 16 that is integrated on

Chromosome 2 (*Tg(Olfr19)Y11*). We previously showed that this transgenic odorant-receptor locus undergoes asynchronous replication[15]. Two-color FISH analysis showed that the transgene was coordinated in its asynchronous replication with the endogenous odorant-receptor loci on Chromosome 2 (Fig. 2*h*), suggesting similarities in the mechanisms governing allele-specific replication timing on different chromosomes.

Here, we present data indicating that randomly monoallelically expressed genes coordinate their asynchronous replication within each chromosome pair. Scattered genes along a given chromosome are coordinated in their asynchronous replication timing in *cis*, leaving unaffected the bulk of the genes (which are synchronously replicated or, in rare instances, asynchronous but imprinted). Asynchronous replication is established early in development[10,11] and maintained in the progeny of individual cells in a clonal manner (ref. 10; Fig. 1*e*–*h* and see Supplementary Tables 1 and 2 online). Randomly monoallelically expressed genes are expressed in different cells of a given cell type or in different cell types. Therefore, coordination of replication timing does not imply coordination of transcription of distinct gene families and is instead a consequence of the early developmental mechanisms that establish asynchronous replication. Each gene family probably makes use of asynchronous replication (and the differences in chromatin structure that it reflects) in the complex gene regulation that characterizes these gene families. In the case of the immunoglobulin genes, we have recently shown that early replication correlates with the allele that will first undergo rearrangement and therefore provides a basis for the establishment of allelic exclusion[10].

X inactivation has been known for decades. Our data indicate that chromosome-pair non-equivalence is also found on autosomes and thus is a general, fundamental property of chromosomes that affects a large number of loci dispersed throughout the genome. The autosomal non-equivalence we observe is similar to that observed with X inactivation, except that a larger fraction of the genes on the X chromosome are affected. Other similarities in the underlying mechanisms of X inactivation and autosomal non-equivalence may emerge with further investigation.

Note: Supplementary information is available on the Nature Genetics website.

Nandita Singh[1]*, Farah A.W. Ebrahimi[1,2]*, Alexander A. Gimelbrant[1], Alexander W. Ensminger[1,2], Michael R. Tackett[1,2], Peimin Qi[1], Joost Gribnau[1] & Andrew Chess[1,2]

**These authors contributed equally to this work.*

[1]Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, Massachusetts 02142, USA. [2]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. Correspondence should be addressed to A.C. (e-mail: chess@wi.mit.edu).

Acknowledgments

We thank H. Cedar, G. Fink, D. Housman, R. Jaenisch, D. Page, C. Cowles and members of A.C.'s laboratory for discussions and comments on the manuscript; H. Higgins for manuscript preparation; G. Paradis for help with fluorescence-activated cell sorting analyses; J. Young for Chromosome 6 BACs, D. Littman for the Cd4 cosmid; R. Lane for the Olfr41 BAC; B. Holdener for the tyrosinase deletion mice; A. Dunn for the CSF2 knock-in mice; P. Soriano for the β-geo transgenic mice; N. Rosenberg for the Abelson leukemia virus–producing lines; D. MacAlpine for advice on immunoprecipitation; S. Gabriel, B. Blumenstiel, M. DeFelice and E. Winchester for help with MALDI-TOF genotyping; and S. Rozen for help with sequencing. This work was supported by grants from the U.S. National Institutes of Health (National Institute on Deafness and Other Communication Disorders) to A.C., F.A.W.E. and M.T. A. W.E. is a Howard Hughes Medical Institute Predoctoral Fellow.

Competing interests statement

The authors declare that they have no competing financial interests.

Received 6 December 2002; accepted 19 January 2003.

1. Lyon, M.F. *Nature* **320**, 313 (1986).
2. Efstratiadis, A. *Curr. Opin. Genet. Dev.* **4**, 265–280 (1994).
3. Chess, A., Simon, I., Cedar, H. & Axel, R. *Cell* **78**, 823–834 (1994).
4. Pernis, B., Chiappino, G., Kelus, A.S. & Gell, P.G. *J. Exp. Med.* **122**, 853–876 (1965).
5. Rajewsky, K. *Nature* **381**, 751–758 (1996).
6. Rhoades, K.L. *et al. Curr. Biol.* **10**, 789–792 (2000).
7. Hollander, G.A. *et al. Science* **279**, 2118–2121 (1998).
8. Held, W., Roland, J. & Raulet, D.H. *Nature* **376**, 355–358 (1995).
9. Rodriguez, I., Feinstein, P. & Mombaerts, P. *Cell* **97**, 199–208 (1999).
10. Mostoslavsky, R. *et al. Nature* **414**, 221–225 (2001).
11. Simon, I. *et al. Nature* **401**, 929–932 (1999).
12. Young, J.M. *et al. Hum. Mol. Genet.* **11**, 535–546 (2002).
13. Zhang, X. & Firestein, S. *Nat. Neurosci.* **5**, 124–133 (2002).
14. Selig, S., Okumura, K., Ward, D.C. & Cedar, H. *EMBO J.* **11**, 1217–1225 (1992).
15. Ebrahimi, F.A., Edmondson, J., Rothstein, R. & Chess, A. *Dev. Dyn.* **217**, 225–231 (2000).

© 2003 Nature Publishing Group http://www.nature.com/naturegenetics