

THE MAMMALIAN BETA GLOBIN ORIGIN OF DNA REPLICATION

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1. ABSTRACT

Initiation of DNA replication is a tightly regulated process aimed to insure that the entire genome is replicated at the appropriate time during each cell cycle. In the human beta-globin locus, replication initiates from a region between the two genes that encode the adult subunit of hemoglobin (the beta-globin initiation region, or IR).

Mammalian beta-globin loci replicate early during the S-phase of the cell cycle in pre-erythroid cells, in which the beta-globin locus is present in a euchromatin form.

However, in cells that do not express globin and in which the locus is heterochromatic, these same loci replicate during the later stages of S-phase. Both early and late replication patterns utilize similar replication initiation regions. These features make the beta-globin locus an attractive model for studying the determinants of replication sites and replication timing, as well as the correlation between gene expression and DNA replication.

Two genomic domains are essential for initiation of DNA replication within the locus: the initiation region (IR), and a 40 kb region upstream of the globin gene cluster known as the locus control region (LCR). The IR meets the genetic requirements for a chromosomal replicator, since it can initiate DNA replication at ectopic sites. The LCR regulates transcriptional activity and chromatin structure, and may act as a determinant of replication timing. This review will summarize recent findings characterizing the sequence requirements for initiation of DNA replication in mammalian beta-globin loci and will discuss the specific influence of the location and the chromosomal environment in regulating DNA replication at the beta-globin IR.

2. THE LOCATION: INITIATION OF DNA REPLICATION FROM THE beta-GLOBIN INITIATION REGION (IR)

2.1. The human beta-globin initiation region (IR)

The beta-globin locus resides on human chromosome 11 and includes five genes that encode the beta-subunit of hemoglobin (Figure 1A). Mutations and

deletions within this locus can result in severe blood disorders, such as anemias and thalassemias. Because of the importance of the beta-globin locus to human health, considerable effort has been directed towards understanding the structure-function relationships within this locus, as well as its chromatin structure and how its gene expression is regulated. Furthermore, the medical interest in this locus has resulted in the development of an extensive repository of point and deletion beta-globin mutants that are available for analysis. The availability of sequence and functional information for the entire locus made this genomic region a particularly attractive model for replication studies.

Replication forks within the human beta-globin locus primarily initiate DNA replication from a replication initiation region (globin IR) between the two adult globin genes, delta and beta. Several approaches were used to define this initiation region. First, Kitsberg *et al.* (1) mapped the direction of replication forks at the globin locus using a method that hinders Okazaki fragment formation by inhibiting protein synthesis and examines the templates of newly replicated leading strands. They identified a single region within the entire human beta-globin locus in which replication forks changed directions between the two adult genes (the initiation region, or IR - Figure 1A). These data were confirmed by fluorescent *in situ* hybridization with cosmids spanning the beta-globin locus, suggesting that the region containing the IR was replicated prior to the regions straddling it (1). Although the *in situ* hybridization method used cosmids, and therefore was of low resolution, the results provided supporting evidence that replication initiated primarily from the IR. Mapping replication initiation sites by the nascent strand abundance method (2) demonstrated that sequences from the IR are enriched in newly replicated, short DNA strands, further suggesting that DNA replication is initiated from within the IR region.

This second analysis confirmed that replication initiated within the IR based on independent leading strand analyses

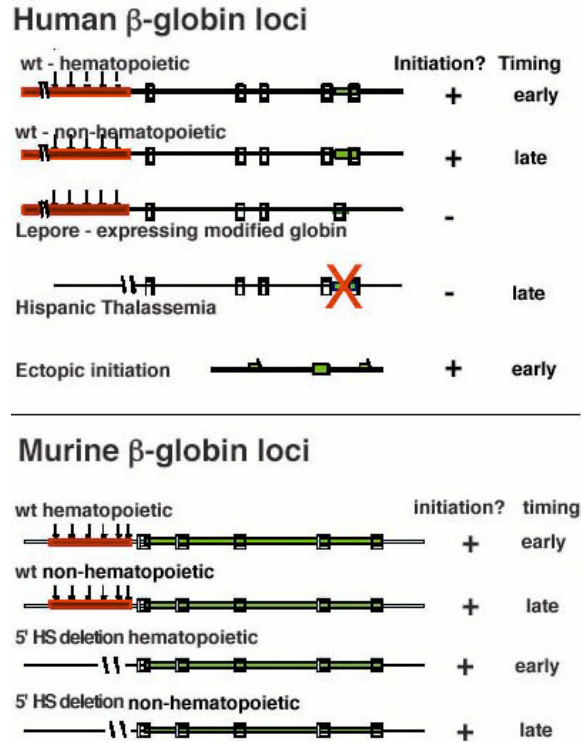


Figure 1. Replication initiation sites in beta globin loci. A. The human beta globin initiation region (IR - green box) resides within the two adult beta globin genes, delta and beta. The locus control region (LCR - red box) contains 5 DNase hypersensitive sites (arrows) and is located 50kb downstream to the IR. An enlargement of the IR is shown. The IR region (empty box) exhibits replicator activity; the central (gray) region is essential for this replicator activity but does not function as a replicator when inserted by itself into ectopic sites. The illustration below the IR shows the starting point for transcription of the beta globin gene (dashed arrow) and the location of the exons (boxes). The location of the following sequence features are indicated: Matrix attachment sites (MAR); Enhancers (Enh); conserved purine-pyrimidine stretches ((RY)n) and a transient DNase hypersensitive site (gray arrow). B. Replication initiation profiles for beta globin loci in various vertebrate species. Initiation sites are shown as green boxes; locus control regions are shown in red boxes; hypersensitive sites are depicted as arrows; gene locations are shown as empty black boxes. The condensed chromatin region at the 5' end of the chicken beta globin locus is shown as an orange box. The human and the murine loci are straddled by olfactory receptor arrays; the chicken locus has olfactory receptor arrays beyond the 3' hypersensitive site and a folate receptor region upstream of the DNase hypersensitive site that marks the boundary of the 5' condensed chromatin region.

and *in situ* hybridizations. A third analysis (3) confirmed that replication is initiated within the IR. This analysis also suggested that a primer pair corresponding to DNA

sequences in the region 5' to the IR could amplify products in short nascent strands, however, other nascent strands analyses did not detect initiation with primers corresponding to DNA sequences located within the same region (2). Since the region located 5' to the IR does not exhibit a switch in the direction of replication forks (1, 2), it is likely that the majority of initiation events in the human beta-globin locus occur within the IR.

In wild-type cells, the human globin locus replicates early in cells of the erythroid lineage that express globin, and late in cells that do not express globin (4, 5). Despite the altered replication timing, origin usage in the human beta-globin locus does not change; DNA replication is initiated at a similar location in erythroid and non-erythroid cell lines (1) Origin usage is also conserved when the human beta-globin locus is transferred into cells originating from other vertebrates: replication initiated within the same IR in murine-human somatic cell hybrids and chicken-human somatic cell hybrids that contained human chromosome 11 (2). Unpublished data from the author's laboratory indicate that when yeast artificial chromosomes harboring the entire (180 kb) human globin locus are transferred to murine cells, the replication initiation profile exhibited resembles that observed in the intact human chromosome 11.

Mapping of the replication initiation profiles in beta-globin loci provided important clues regarding the roles of specific DNA sequences in directing initiation sites in mammalian cells. Kitsberg *et al.* (1) demonstrated that the naturally occurring Lepore deletion, which removes the IR, results in passive replication of the locus from an outside origin. These data suggested that the initiation region between the two adult genes contained information essential for initiation (see section below). Analysis of the Hispanic thalassemia deletion suggested that initiation of DNA replication from the IR requires sequences located 50 kb 5' to the IR, which includes the locus control region (LCR) (2). Interestingly, in the Lepore chromosome, replication forks traverse the locus from the 5' to the 3' end (1), while in the Hispanic thalassemia, replication forks traverse the locus from the 3' to the 5' (2). These observations suggest that a replication origin, active in the Lepore chromosome, resides in the region deleted by the Hispanic thalassemia. The altered initiation patterns in the two deletions imply that replication initiation requires specific information harbored within genetic elements that may coincide with the origin; in addition, replication initiation may also require interaction with other distal elements.

2.2. Is the IR a replicator?

The replicon model of Jacob, Brenner and Cuzin (6) proposed that all cells regulate DNA replication through the interaction of *cis* acting DNA sequences called "replicators" with *trans*-acting initiation factors called "initiators". Although replicators often colocalize with replication origins, the two terms are not synonymous: replicators are defined genetically, based on their ability to confer initiation of DNA replication, whereas replication

origins are defined biochemically, as the sites from which replication initiates.

In mammalian cells, DNA sequences meeting the biochemical criteria of origins typically do not replicate extrachromosomally, unless they are linked to viral replicons or nuclear retention signals (7). In addition, different biochemical strategies designed to identify initiation regions in mammalian cells have sometimes generated conflicting data (7, 8). These observations led to a controversy regarding whether replicators exist in mammals and whether replication in mammalian cells initiates from fixed chromosomal sites. Analyses of the replication profiles in the human beta-globin locus suggested that in this region, replication initiates consistently within the IR (1,2). Moreover, the observed absence of initiation from beta-globin loci harboring the Lepore deletion, which do not contain the IR (1), suggested that replication required genetic information uniquely supplied by the deleted region. Hence, the globin IR may function as a mammalian genetic replicator.

If the IR contains essential information that is required for replication initiation, it might be able to initiate DNA replication when transferred to a genomic region that lacks inherent replicator activity. To test this hypothesis, the globin IR was transferred to regions in the simian genome that contained no replication origins. A recombinase-mediated procedure was used to transfer putative replicator sequences into fixed chromosomal sites (9). The IR was able to initiate DNA replication within these simian regions, which hitherto did not exhibit initiation activity, suggesting that this region meets the requirements for a genetic replicator. The ability to confer initiation of DNA replication was unique to some sequences within the globin IR, as other sequences of similar sizes did not confer initiation when inserted into the same constant sites in simian cells (9). The IR was also used in human artificial chromosomes that seem to be able to replicate extrachromosomally in human cells (10). These data indicate that initiation of DNA replication in mammalian cells requires specific sequence information located in replicator sequences, similar to what is observed in prokaryotes and unicellular eukaryotes.

The ectopic initiation assay further facilitated identification of sequences essential for replicator activity within the IR. Deletion analyses (9) suggest that the beta-globin replicator includes a core element containing the initiation region, but this core element is not sufficient for initiation. The core can be complemented by two auxiliary elements, either one of which could support initiation from the core (Figure 1B). The core region contains several features thought to be associated with replication origins: AT-rich tracts, matrix attachment sites and DNase hypersensitive sites (11-14). However, these features are also common in DNA sequences that do not serve as replication origins, and the significance of their presence in the IR is not clear. Interestingly, evolutionary analyses had shown a remarkably conserved region of alternate purine-pyrimidine nucleotides within the core replicator (15), whereas the region surrounding the IR does not

exhibit any extraordinary bias in mutation rate (16). These data suggest that the conserved sequences may play an important role in cellular metabolism, possibly in replication, yet the exact sequence requirements for initiation remain to be elucidated. Recent unpublished observations from the author's laboratory suggest that the globin IR contains a few regions that can potentially act as replicators, and that sequence elements within each of these regions cooperate to determine the site of initiation of DNA replication. Because the redundant replicator sequences are adjacent to each other, biochemical detection methods cannot establish whether each of these putative replicators actually initiate DNA replication in mammalian cells, or whether one of these sites is dominant over the other in the native globin locus. A detailed analysis of ectopic replicator activity is likely to shed light on the sequence requirements for initiation of DNA replication in this locus.

2.3. The globin IR in other vertebrates: conservation and divergence

Replication initiates from fixed sites in vertebrate genomes, but it is not known whether the initiation sites are conserved between species. Since human beta-globin loci initiate DNA replication from the IR when transferred into a murine or chicken environment, it is likely that the replication machineries in all vertebrates recognize similar replicator sequences as markers for replication initiation sites. However, this conservation does not necessarily imply that replication initiates from homologous sequences in homologous loci. To date, the replication initiation profile of the beta-globin locus has been determined three vertebrate species – human, murine and chicken. Data from these initiation analyses suggest that replication initiation sites exhibit a large degree of dissimilarity.

Replication initiation patterns were mapped in murine beta-globin loci in murine embryonic stem (ES) cells and in somatic murine cell lines (17). Murine chromosomes share a high degree of sequence similarity with human chromosomes and the organization of the murine and human beta-globin loci is very similar (Figure 1B). Unlike the restricted initiation observed in the human beta-globin locus, replication in murine cells initiates from multiple origins throughout the region coding for the beta-like globin genes. A genetic analysis supported this conclusion, as deletion of the region homologous to the human globin IR from the murine globin locus produced chromosomes in which replication initiated from the other origins (Figure 2). These analyses were performed using both replication fork direction mapping and nascent strand abundance assays. Although the studies did not enumerate the replication initiation sites and replicators in the murine betaglobin locus, these observations suggested that the information required for replication initiation in the murine locus does not lie exclusively within the region between the adult globin genes.

Although the replication initiation sites are divergent, the replication timing patterns are conserved between the human and murine loci. Similar to observations in human cells, the murine beta-globin locus was found to replicate late during S-phase in cells that do

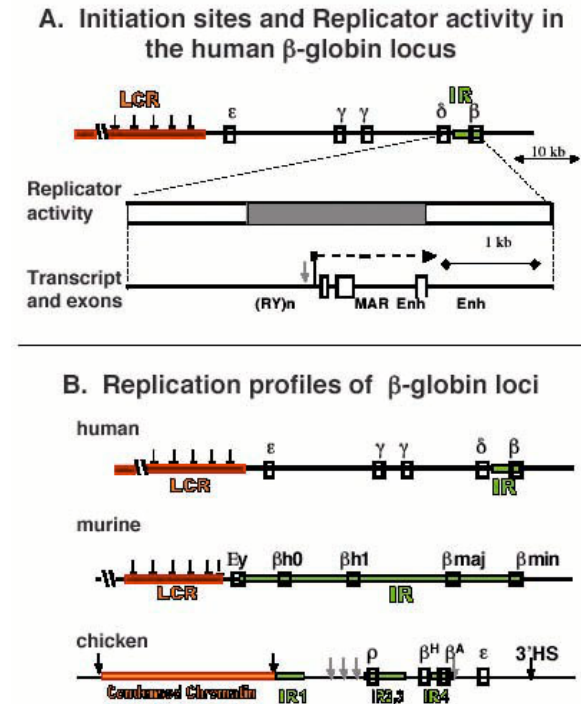


Figure 2. The effect of the cellular and chromosomal environment on the site and timing of initiation of DNA replication. Initiation sites are shown as green boxes; locus control regions are shown as red boxes; exons are shown as empty boxes; FLP recombinase recognition sites are shown as yellow arrows. The human beta globin locus initiates DNA replication from an 8 kb IR regardless of cell type but replicates early during S-phase in hematopoietic cells and late in non-hematopoietic cells. Deletion of the initiation region (Lepore deletion) does not allow initiation of DNA replication within the locus. An LCR deletion also exhibits no initiation within the locus; the locus replicates very late, even in erythroid cells. Ectopic initiation from the IR can proceed without the LCR. This initiation occurs early during the S-phase of the cell cycle. The murine loci exhibit the same tissue specificity of replication timing but the LCR deletion does not affect initiation sites.

not express globin (such as ES cells) and early in globin-expressing cells of the hematopoietic lineage. Origin usage did not differ in cells that replicated the locus at different times during S-phase, which is also similar to what was observed in human cells (Figure 2).

The initiation profile in the chicken beta-globin region was recently characterized (17a). The chicken beta-globin locus exhibits a different organization of the beta-globin-like genes and its regulatory elements are found in different locations, as compared to the human beta-globin locus (Figure 2A). The chicken beta-globin IR contains four replication initiation sites, one of which is situated in a boundary region between the beta-globin region and an adjacent erythroid-specific folate receptor region. Unlike the conserved replication timing patterns observed in murine and human cells, the chicken locus

replicates early in all cell types, including cells that do not express globin.

The divergence of replication initiation sites contrasts with the fact that human chromosomes transferred to a murine or chicken environment continue to initiate DNA replication according to the human program (2). This indicates that the murine and chicken replication machinery is capable of deciphering the determinants of replication timing from human chromosomes, suggesting that these signals are similar within vertebrates. The divergence in replication initiation sites suggests that information that determines replication initiation sites is embedded at the level of DNA sequence and chromatin structure, and that it is not altered by the cellular environment. On the other hand, the replication timing program is determined by the cellular environment and the differentiation status of cells and this program is conserved in mammalian cells. Such conservation suggests that modulation of replication timing may play an important role in the regulation of cellular metabolism.

3. THE CHROMOSOMAL ENVIRONMENT AND ITS ROLE IN DETERMINING THE SITE AND TIMING OF DNA REPLICATION

The chromosomal and cellular environments play important roles in determining replicator function. As mentioned above, differentiation into erythroid cells alters the timing of DNA replication at the human and murine beta-globin loci. Origin usage can also depend on interactions that occur at a distance. For example, initiation from the human beta-globin replication origin in its native site requires a region located 50 kb upstream. The same region is required for early replication in erythroid cells. Other loci are subject to similar mechanisms of regulation of replication timing during differentiation (18,19) and distant sequences have also been shown to be required for origin specifications in other mammalian loci, such as the Chinese hamster DHFR (20). These observations suggest that the capacity to initiate replication from a specific origin at a particular time might depend on its location relative to distant "auxiliary" elements, and on other factors, such as the activation of gene expression.

3.1. Replication timing and gene expression

In the mammalian genome, initiation of DNA replication from distinct chromosomal domains occurs in a strictly conserved order (21). Aberrations in replication timing correlate with a loss of imprinting (22,23), activation of cell cycle checkpoints (24), and genomic instability (25,26). As mentioned above, the human beta-globin locus utilizes a consistent initiation region for early replication in pre-erythroid cells that express globin and for late replication in cells that do not express globin (4,5).

The globin locus is embedded within a cluster of olfactory receptor genes at the telomeric end of human chromosome 11 (27). Interestingly, this region exhibits asynchronous replication - one of the chromosomal alleles replicates early during the S-phase of the cell cycle while

the other replicates late (28). The beta-globin locus forms a special zone within this region, which exhibits synchronous replication of both alleles regardless of the replication status of the surrounding region. In non-erythroid cells, which do not express globin, both alleles of the beta-globin locus replicate late. In erythroid cells, this synchronous zone expands to include the neighboring olfactory receptor locus and exhibits earlier replication (28). A similar correlation between activation of silent genes and a switch to early replication was also observed in the immunoglobulin (18), CD8 (19), and T cell receptor (29) loci.

The observed switch in replication timing during erythroid differentiation is consistent with the notion that early replication correlates with active transcription, whereas untranscribed heterochromatin replicates late. Although this concept is not consistent with studies in yeast (30,31), a correlation between gene expression and early replication was recently demonstrated for the *Drosophila* genome (32), suggesting that such a correlation may be a metazoan feature. However, inactivation of globin transcription in somatic cell hybrids harboring the human beta-globin locus did not result in late replication or in loss of origin activity (2,33). Although transcription is absent, these loci are maintained in an open chromatin conformation in erythroid cells. Similarly, early replication was observed in murine chromosomes that harbor a deletion of the LCR hypersensitive sites; such cells exhibit very low levels of globin gene expression, but still show chromatin decondensation (17). These observations suggest that early replication at the beta-globin locus correlates with the ability to decondense chromatin, not with actual transcription.

3.2. Does origin choice require activation of cell cycle checkpoints?

In contrast to replication timing, which can change in response to differentiation and activation of gene expression, replication initiation sites in mammalian cells are not known to exhibit tissue specificity. This observation has been confirmed in human, murine and chicken globin loci (see above). However, evidence from other metazoans suggests that these sites do change in early development. For example, replication initiates randomly during the early stages of *Xenopus* development and becomes restricted to preferred sites at the mid-blastula transition, which coincides with the onset of transcription and chromatin remodeling (34). Interestingly, undifferentiated wild-type murine embryonic stem (ES) cells initiate DNA replication at identical sites as do somatic differentiated cells. ES cells are collected from the inner cell mass of the murine blastula, and do not exhibit the prolonged “gap” phases (G1 and G2) of the cell cycle.

Rather, these cells appear to oscillate between S-phase and M-phase, similar to other rapidly proliferating embryonic cells. ES cells also differ from somatic tissue in that they do not activate p53-mediated controls that induce cell cycle arrest in response to DNA damage (35). The similarity in origin usage between ES cells and somatic cells suggests that origin specification does not require the activation of

the somatic cell cycle checkpoints. Conversely, the above observations are consistent with the possibility that specification of replication origins may correlate with chromatin remodeling. Unlike *Xenopus* embryos, which remodel chromatin after 12 divisions, murine embryos undergo chromatin remodeling and initiation of transcription prior to the 4-cell stage (36,37). Therefore, transcription and chromatin remodeling are complete in ES cells. Since ES cells initiate DNA replication from specific origins, we can conclude that origin specification may require chromatin remodeling. However, such chromatin remodeling occurs prior to and independently from the establishment of the prolonged G1 phase and some of the cell cycle controls that characterize somatic differentiated cells.

3.3. The role of the locus control region

In the human beta-globin locus, deletion of 5' DNase hypersensitive sites and upstream sequences in Hispanic Thalassemia prevents replication initiation from the normal origin (2) (Figure 2). This deletion leads to the loss of a 40 kb region upstream of the globin gene cluster, including a series of DNase I hypersensitive sites known collectively as the locus control region (LCR). The human LCR contains five DNase hypersensitive sites, which contribute to gene expression, but are not required for establishing or maintaining an open chromatin conformation in a developmentally specific manner ((38); for review see (39)). Deletion of the LCR resulted in a late replication pattern of the entire beta-globin locus, even in erythroid cells (38). These observations suggested that in addition to its role as a transcriptional enhancer, the LCR facilitates the initiation of DNA replication from the IR.

Analysis of ectopic sites suggests that the LCR plays a role in establishing replication timing. For example, LCR sequences inserted in various random locations within murine chromosomes can confer tissue-specific replication timing of adjacent globin genes (28). Tissue-specific replication timing was not conferred by an assembly of the LCR hypersensitive site “cores”, which are the regions within the hypersensitive site that bind to erythroid-specific and general transcription factors (28). On the other hand, deletions of the regions that include all the hypersensitive sites in the human locus and the murine loci neither altered replication timing nor changed the replication initiation profiles (17,40). It should be noted, however, that these LCR deletions also exhibited tissue-specific chromatin decondensation of the globin genes and a normal pattern of gene expression, although the magnitude of gene expression was reduced (41). These deletions, therefore, did not confer the Hispanic thalassemia phenotype. These observations suggest that sequences outside the LCR hypersensitive sites, but within the region deleted in Hispanic thalassemia, may play a critical role in origin specification and replication timing at the human beta-globin locus.

Replicator assays at ectopic sites suggest that the IR, and fragments of the IR, can initiate replication at simian ectopic sites in the presence or absence of the LCR.

To date, LCR-independent replication at ectopic sites has been shown to initiate early during the S-phase of the cell cycle, and insertion of LCR sequences did not alter replication timing (9). These observations are consistent with the hypothesis that initiation of DNA replication from the IR does not require direct interaction with the LCR, but that other sequences may substitute for the LCR in providing an environment that allows initiation of DNA replication early during S-phase. These data appear to conflict with the observation that random insertions of beta-globin LCR-promoter-gene fusions in transgenic mice preserve the tissue-specific nature of replication timing (28). Moreover, these findings also seem to conflict with the previously observed requirement for the LCR to initiate DNA replication in the native locus. One possible way to reconcile these observations is the hypothesis that the LCR plays a role in replication timing only in the context of late-replicating chromatin domains. Therefore, the LCR may play a role in establishing an environment that permits initiation of DNA replication in the native locus, which is embedded in the olfactory receptor array. However, the native locus would not be required to facilitate initiation of DNA replication in transgenes containing replicator sequences from the human beta-globin IR at constitutively expressed, early-replicating sites.

If the primary role of the LCR is to establish a replication timing domain, why does the Hispanic thalassemia deletion fail to initiate DNA replication from the IR? The answer may relate to competition between late initiating replication origins and earlier established replication forks. In yeast, late replicating origins removed from their chromosomal context may retain their original replication timing, but a detailed dissection of these origin fragments demonstrates that the sequences determining replication timing are distinct from those that dictate replication initiation (42). Late replicating origins, placed near earlier replicating domains, sometimes fail to replicate in yeast, unless the earlier replicating origin is inactivated. Similarly, removal of the LCR may cause late replication of the entire locus, and allow replication forks from adjacent replication origins to reach the locus before initiation from the IR occurs.

To date, all studies of LCR mutants and ectopic insertions have exhibited a correlation between early replication and a decondensed chromatin conformation, although this has not necessarily been correlated with gene expression *per se* (2,40). One intriguing possibility that might explain this correlation is that late replication of condensed chromatin contributes to the preservation of the condensed state through a positive feedback loop that depends on the lack of histone acetylation during late S-phase (21). If this hypothesis is correct, tissue-specific differentiation that leads to chromatin decondensation at the globin IR should also lead to earlier replication through the recruitment of a chromatin modifier. Chromatin modifiers may render the locus more accessible to components of the pre-replication complex, a complex of proteins which binds to replication origins prior to initiation of DNA replication. In heterochromatic regions, lack of

these chromatin modifiers perpetuates the late replication status. In Hispanic thalassemia, the locus is either inaccessible to the pre-replication complex, or is scheduled to replicate later than adjacent replication origins, and is therefore replicated passively by replication forks emanating from outside the locus, as discussed above.

Further characterization of the dynamics of gene activation and replication timing are likely to shed light on the complex mechanisms by which cells determine which sequence will initiate DNA replication at a specific time in the cell cycle.

4. CONCLUSIONS

The origin of DNA replication at the human beta-globin locus is an example of a tissue-specific replication initiation region. Replication initiates from the globin IR regardless of the timing of DNA replication, but replication timing is tissue-specific and correlates with the condensation status of chromatin. Two sequence elements play a role in determining the site and timing of initiation of DNA replication in the beta-globin locus. The IR, or replication origin, functions as a replicator, since its presence is required for initiation at the native locus and it is able to initiate DNA replication at ectopic sites. This replicator contains redundant sequences that cooperate in order to initiate DNA replication. The LCR, a distal element, primarily seems to set the timing of DNA replication. The LCR is required for replication within the native locus, but not at ectopic sites, suggesting that this element does not interact directly with the IR, but rather renders the globin locus permissive for replication through modification of chromatin. Further dissection of the roles of the IR and LCR in the globin locus will help to elucidate the determinants of replication sites and timing in mammalian cells.

5. REFERENCES

1. Kitsberg, D., S. Selig, I. Keshet and H. Cedar: Replication structure of the human beta-globin gene domain. *Nature* 366, 588-590 (1993)
2. Aladjem, M. I., M. Groudine, L. L. Brody, E. S. Dieken, R. E. K. Fournier, G. M. Wahl and E. M. Epner: Participation of the human beta globin locus control region in initiation of DNA replication. *Science* 270, 815-819 (1995)
3. Kamath, S. and M. Leffak: Multiple sites of replication initiation in the human beta-globin gene locus. *Nucleic Acids Res* 29, 809-817 (2001)
4. Epner, E., W. C. Forrester and M. Groudine: Asynchronous DNA replication within the human beta-globin gene locus. *Proc Natl Acad Sci USA* 85, 8081-8085 (1988)
5. Dhar, V., A. I. Skoultschi and C. L. Schildkraut: Activation and repression of a beta-globin gene in cell hybrids is accompanied by a shift in its temporal

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replication. *Mol Cell Biol* 9, 3524-3532 (1989)

6. Jacob, F., J. Brenner and F. Cuzin: On the regulation of DNA replication in bacteria. *Cold Spring Harbor Dym Quant Biol* 288, 329 (1964)

7. DePamphilis, M. L.: The search for origins of DNA replication. *Methods* 13, 211-219 (1997)

8. Bogan, J. A., D. A. Natale and M. L. Depamphilis: Initiation of eukaryotic DNA replication: Conservative or liberal? *J Cell Physiol* 184, 139-150 (2000)

9. Aladjem, M., L.-W. Rodewald, J. L. Kolman and G. M. Wahl: Genetic dissection of a mammalian replicator in the human beta-globin locus. *Science* 281, 1005-1009 (1998)

10. Henning, K. A., E. A. Novotny, S. T. Compton, X. Y. Guan, P. P. Liu and M. A. Ashlock: Human artificial chromosomes generated by modification of a yeast artificial chromosome containing both human alpha satellite and single-copy DNA sequences. *Proc Natl Acad Sci USA* 96, 592-597 (1999)

11. Boulikas, T.: Homeodomain protein binding sites, inverted repeats, and nuclear matrix attachment regions along the human beta-globin gene complex. *J Cell Biochem* 52, 23-36 (1993)

12. Boulikas, T.: Common structural features of replication origins in all life forms. *J Cell Biochem* 60, 297-316 (1996)

13. Djeliova, V., G. Russev and B. Anachkova: Dnase i sensitive site in the core region of the human beta-globin origin of replication. *J Cell Biochem* 87, 279-283 (2002)

14. Djeliova, V., G. Russev and B. Anachkova: Dynamics of association of origins of DNA replication with the nuclear matrix during the cell cycle. *Nucleic Acids Res* 29, 3181-3187 (2001)

15. Fullerton, S. M., J. Bond, J. A. Schneider, B. Hamilton, R. M. Harding, A. J. Boyce and J. B. Clegg: Polymorphism and divergence in the beta-globin replication origin initiation region. *Mol Biol Evol* 17, 179-188 (2000)

16. Francino, M. P. and H. Ochman: Strand symmetry around the beta-globin origin of replication in primates. *Mol Biol Evol* 17, 416-422 (2000)

17. Aladjem, M. I., L. W. Rodewald, C. M. Lin, S. Bowman, D. M. Cimborra, L. L. Brody, E. M. Epner, M. Groudine and G. M. Wahl: Replication initiation patterns in the beta-globin loci of totipotent and differentiated murine cells: Evidence for multiple initiation regions. *Mol Cell Biol* 22, 442-452 (2002)

17a. Prioleau, M.N., Gendron, M.C., Hyrien, O.: Replication of the chicken beta-globin locus: early-firing origins at the 5' HS4 insulator and the rho- and betaA-globin genes show opposite epigenetic modifications. *Mol*

Cell Biol 23, 3536-49 (2003)

18. Ermakova, O. V., L. H. Nguyen, R. D. Little, C. Chevillard, R. Riblet, N. Ashouian, B. K. Birshtein and C. L. Schildkraut: Evidence that a single replication fork proceeds from early to late replicating domains in the igh locus in a non-b cell line. *Mol Cell* 3, 321-330 (1999)

19. Hibbard, M. K., S. Strehl and M. Lalande: Replication timing of cd4 and cd8 in single-positive peripheral blood lymphocytes. *Cell Immunol* 198, 61-68 (1999)

20. Kalejta, R. F., X. Li, L. D. Mesner, P. A. Dijkwel, H. B. Lin and J. L. Hamlin: Distal sequences, but not ori-beta/obr-1, are essential for initiation of DNA replication in the chinese hamster dhfr origin. *Mol Cell* 2, 797-806 (1998)

21. Gilbert, D. M.: Replication timing and transcriptional control: Beyond cause and effect. *Curr Opin Cell Biol* 14, 377-383 (2002)

22. Hansen, R. S., T. K. Canfield, A. D. Fjeld and S. M. Gartler: Role of late replication timing in the silencing of x-linked genes. *Hum Mol Genet* 5, 1345-1353 (1996)

23. Hansen, R. S., R. Stoger, C. Wijmenga, A. M. Stanek, T. K. Canfield, P. Luo, M. R. Matarazzo, M. D'Esposito, R. Feil, G. Gimelli, C. M. Weemaes, C. D. Laird and S. M. Gartler: Escape from gene silencing in icf syndrome: Evidence for advanced replication time as a major determinant. *Hum Mol Genet* 9, 2575-2587 (2000)

24. Loupart, M. L., S. A. Krause and M. S. Heck: Aberrant replication timing induces defective chromosome condensation in drosophila orc2 mutants. *Curr Biol* 10, 1547-1556 (2000)

25. Amiel, A., A. Elis, S. Sherker, E. Gaber, Y. Manor and M. D. Fejgin: The influence of cytogenetic aberrations on gene replication in chronic lymphocytic leukemia patients. *Cancer Genet Cytogenet* 125, 81-86 (2001)

26. Smith, L., A. Plug and M. Thayer: Delayed replication timing leads to delayed mitotic chromosome condensation and chromosomal instability of chromosome translocations. *Proc Natl Acad Sci USA* 98, 13300-13305 (2001)

27. Bulger, M., J. H. von Doorninck, N. Saitoh, A. Telling, C. Farrell, M. A. Bender, G. Felsenfeld, R. Axel and M. Groudine: Conservation of sequence and structure flanking the mouse and human beta-globin loci: The beta-globin genes are embedded within an array of odorant receptor genes. *Proc Natl Acad Sci USA* 96, 5129-5134 (1999)

28. Simon, I., T. Tenzen, R. Mostoslavsky, E. Fibach, L. Lande, E. Milot, J. Gribnau, F. Grosveld, P. Fraser and H. Cedar: Developmental regulation of DNA replication timing at the human beta globin locus. *Embo J* 20, 6150-6157 (2001)

29. Mostoslavsky, R., N. Singh, T. Tenzen, M. Goldmit, C. Gabay, S. Elizur, P. Qi, B. E. Reubinoff, A. Chess, H.

Cedar and Y. Bergman: Asynchronous replication and allelic exclusion in the immune system. *Nature* 414, 221-225 (2001)

30. Raghuraman, M. K., E. A. Winzeler, D. Collingwood, S. Hunt, L. Wodicka, A. Conway, D. J. Lockhart, R. W. Davis, B. J. Brewer and W. L. Fangman: Replication dynamics of the yeast genome. *Science* 294, 115-121 (2001)

31. Kim, S. M., D. D. Dubey and J. A. Huberman: Early replicating heterochromatin. *Genes and Development* 17, 330-335 (2003)

32. Schubeler, D., D. Scalzo, C. Kooperberg, B. Van Steensel, J. Delrow and M. Groudine: Genome-wide DNA replication profile for drosophila melanogaster: A link between transcription and replication timing. *Nat Genet* (2002)

33. Kim, C. G., E. M. Epner, W. C. Forrester and M. Groudine: Inactivation of the human beta-globin gene by targeted insertion into the beta-globin locus control region. *Genes Dev* 6, 928-938 (1992)

34. Hyrien, O., C. Maric and M. Mechali: Transition in specification of embryonic metazoan DNA replication origins. *Science* 270, 994-997 (1995)

35. Aladjem, M. I., B. T. Spike, L. W. Rodewald, T. J. Hope, M. Klemm, R. Jaenisch and G. M. Wahl: Es cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. *Current Biol* 8, 145-155 (1998)

36. Majumder, S., Z. Zhao, K. Kaneko and M. L. DePamphilis: Developmental acquisition of enhancer function requires a unique coactivator activity. *EMBO J* 16, 1721-1731 (1997)

37. Wiekowski, M., M. Miranda, J. Y. Nothias, M. L. DePamphilis: Changes in histone synthesis and modification at the beginning of mouse development correlate with the establishment of chromatin mediated repression of transcription. *J Cell Sci* 1147-1158 (1997)

38. Forrester, W. C., S. Takegawa, T. Papayannopoulou, G. Stamatoyannopoulos and M. Groudine: Evidence for a locus activation region: The formation of developmentally stable hypersensitive sites in globin-expressing hybrids. *Nuc Acids Res* 15, 10159-10177 (1987)

39. Li, Q., K. R. Peterson, X. Fang and G. Stamatoyannopoulos: Locus control regions. *Blood* 100, 3077-3086 (2002)

40. Cimbor, D. M., D. Schubeler, A. Reik, J. Hamilton, C. Francastel, E. M. Epner and M. Groudine: Long-distance control of origin choice and replication timing in the human beta-globin locus are independent of the locus control region. *Mol Cell Biol* 20, 5581-5591 (2000)

41. Reik, A., A. Telling, G. Zitnik, D. Cimbor, E. Epner and M. Groudine: The locus control region is necessary for gene expression in the human beta-globin locus but not the maintenance of an open chromatin structure in erythroid cells. *Mol Cell Biol* 18, 5992-6000 (1998)

42. Friedman, K. L., J. D. Diller, B. M. Ferguson, S. V. Nyland, B. J. Brewer and W. L. Fangman: Multiple determinants controlling activation of yeast replication origins late in s phase. *Genes Dev* 10, 1595-1607 (1996)

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