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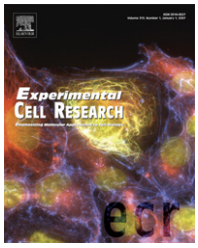

Research Article

Discrete functional elements required for initiation activity of the Chinese hamster dihydrofolate reductase origin beta at ectopic chromosomal sites

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ABSTRACT

The Chinese hamster dihydrofolate reductase (DHFR) DNA replication initiation region, the 5.8 kb ori-beta, can function as a DNA replicator at random ectopic chromosomal sites in hamster cells. We report a detailed genetic analysis of the DiNucleotide Repeat (DNR) element, one of several sequence elements necessary for ectopic ori-beta activity. Deletions within ori-beta identified a 132 bp core region within the DNR element, consisting mainly of dinucleotide repeats, and a downstream region that are required for ori-beta initiation activity at non-specific ectopic sites in hamster cells. Replacement of the DNR element with *Xenopus* or mouse transcriptional elements from rDNA genes restored full levels of initiation activity, but replacement with a nucleosome positioning element or a viral intron sequence did not. The requirement for the DNR element and three other ori-beta sequence elements was conserved when ori-beta activity was tested at either random sites or at a single specific ectopic chromosomal site in human cells. These results confirm the importance of specific *cis*-acting elements in directing the initiation of DNA replication in mammalian cells, and provide new evidence that transcriptional elements can functionally substitute for one of these elements in ori-beta.

Introduction

More than 40 years ago Jacob et al. [1] proposed a DNA replicon model which led to the discovery of replicators from bacteria to mammals [2,3]. A replicator is a *cis*-acting genetic element that directs replication initiation to occur at a specific location recognized by a *trans*-acting initiator. Budding yeast *Saccharomyces cerevisiae* use DNA replicators that contain a short consensus sequence that interacts with the origin recognition complex (ORC) [4]. Genetic, biochemical, and physical mapping of origins of DNA replication on mammalian chromosomes suggests the existence of replicators that may specify DNA replication initiation sites in mammalian cells [5–10]. Moreover, ORC and its role in the initiation of DNA replication are conserved from yeast to mammals, indicating that mammalian replicators might share some features with those of budding yeast [11–19]. However, unlike replicators in budding yeast, the *cis*-acting sequence elements that

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contribute to initiation activity of mammalian replicators have no consensus sequence except for a small AT-rich sequence bias [2,3,20].

To identify a chromosomal replicator in mammalian cells, an origin DNA fragment is placed at an ectopic chromosomal site and assayed for its capacity to direct initiation of replication at the ectopic site. To achieve this, two general strategies have been used. Using a non-specific integration system, the 5.8 kb Chinese hamster DHFR origin beta (ori-beta) [8,9] and the 1.2 kb human LaminB2 origin [21] showed replication initiation activity at multiple chromosomal sites in mammalian cells. These functioned as active replicators in pooled stably transfected cells or individual cell clones, although DNA replication activity varied at different chromosomal sites. The second strategy employed FLP- or Cre-mediated specific recombination integration systems to introduce the 2.4 kb human c-*myc* origin [7,10] and the 2.6 and 3.2 kb human beta-globin origins [6,20,22] into unique ectopic chromosomal sites in a human cell line and a mouse cell line, respectively. The c-*myc* and beta globin origins serve as replicators at their specific ectopic chromosomal sites. Whether the two different strategies are equally effective in identifying mammalian replicators has so far not been validated by directly comparing them with the same mammalian origin.

One of the obstacles to understanding mammalian origins is the lack of identifiable sequence homology between different origins, but even in budding yeast replicators, some elements are not conserved, e.g. the B2 element in ARS1 [23] and the binding site for the Abf1 transcription factor in the ARS1 replicator [24–28]. Interestingly, an Sp1 binding site can functionally replace the Abf1 site in ARS1 [29], suggesting that yeast replicators lacking an Abf1 site contain other elements that may serve the same functional role in directing initiation of replication. Moreover, these results suggest a hypothesis that may explain the DNA sequence diversity among mammalian replicators and that can be experimentally addressed.

The mammalian replicators characterized so far are composed of multiple sequence elements, of which several are required for initiation activity of the replicator. Five sequence elements identified within the 5.8 kb DHFR ori-beta fragment are each necessary, but not individually sufficient, for full initiation activity of ori-beta at ectopic locations in Chinese hamster cells [8,9]. These include a 4 bp GGCC within a GGGCCC palindrome within the peak of initiation activity, an AT-rich element (AT), a CA+GA dinucleotide repeat element (DNR), a region of bent DNA, and a binding site for the 60 kDa Replication Initiation Protein (RIP60) (Fig. 1). A sixth element (not shown) was dispensable for activity [8]. Since each mutation caused a loss of ori-beta activity while the other

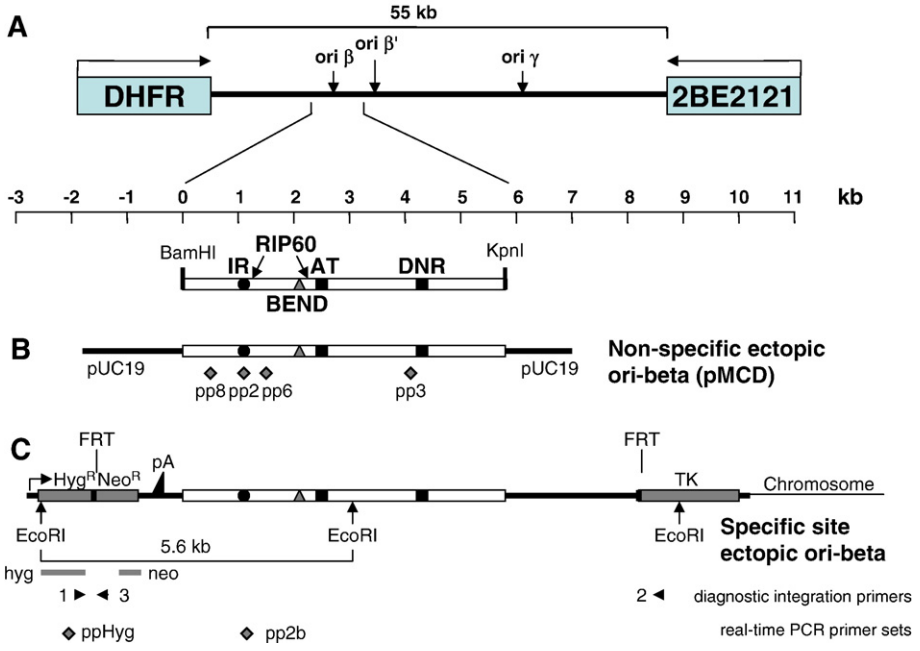

Fig. 1 – The DHFR origin beta at endogenous and ectopic locations. (A) Diagram of the endogenous DHFR ori-beta IR in hamster cells. Preferred start sites of DNA replication (ori-β, ori-β′ , and ori γ) within a 55-kb initiation zone between the genes DHFR and 2BE2121 are indicated. The 5.8 kb DNA fragment containing ori-beta extends from the *Bam*HI (nucleotide position 1) to the *Kpn*I restriction enzyme site. Previously identified [8,9] functional elements of ori-beta are indicated: IR, initiation region; RIP60, 60 kDa replication initiation protein binding sites; BEND, sequence-induced stable bend in the DNA; AT, $(AT)_n$ repeats and AT-rich sequences; DNR, GA+CA dinucleotide repeat element. (B) 5.8 kb ori-beta sequence cloned into pUC19 (pMCD) and integrated at non-specific ectopic locations in DR12 and HeLa cells. Locations of pp8, pp2, pp6 and pp3 PCR primer sites are indicated by gray diamonds. (C) 5.8 kb ori-beta sequence integrated at the FRT site in HeLa 406 cells [10]. The locations of the FRT sites, hygromycin–neomycin resistance fusion gene (Hyg^R Neo^R) with transcription start site (bent arrow), poly-adenylation and transcription termination site (pA), and thymidine kinase (TK) gene are indicated. The 5.6 kb *Eco*RI fragment used for Southern blot analysis, and diagnostic PCR primers 1, 2, and 3 for specific site integration are shown. The target sites for ppHyg and pp2b are indicated with gray diamonds.