Journal of Biotechnology 168 (2013) 174–184

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

A variable fork rate affects timing of origin firing and S phase dynamics in *Saccharomyces cerevisiae*

Adriana Supady[a,1], Edda Klipp[a], Matteo Barberis[a,b,*]

[a] *Institute for Biology, Theoretical Biophysics, Humboldt University Berlin, Invalidenstraße 42, 10115 Berlin, Germany*
[b] *Synthetic Systems Biology and Nuclear Organization, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands*

ARTICLE INFO

Article history:
Received 21 December 2012
Received in revised form 23 May 2013
Accepted 27 June 2013
Available online 9 July 2013

Keywords:
DNA replication
Origin firing
Replication timing
Replication fork rate
Firing efficiency
Budding yeast

ABSTRACT

Activation (in the following referred to as firing) of replication origins is a continuous and irreversible process regulated by availability of DNA replication molecules and cyclin-dependent kinase activities, which are often altered in human cancers. The temporal, progressive origin firing throughout S phase appears as a characteristic replication profile, and computational models have been developed to describe this process. Although evidence from yeast to human indicates that a range of replication fork rates is observed experimentally in order to complete a timely S phase, those models incorporate velocities that are uniform across the genome. Taking advantage of the availability of replication profiles, chromosomal position and replication timing, here we investigated how fork rate may affect origin firing in budding yeast. Our analysis suggested that patterns of origin firing can be observed from a modulation of the fork rate that strongly correlates with origin density. Replication profiles of chromosomes with a low origin density were fitted with a variable fork rate, whereas for the ones with a high origin density a constant fork rate was appropriate. This indeed supports the previously reported correlation between inter-origin distance and fork rate changes. Intriguingly, the calculated correlation between fork rate and timing of origin firing allowed the estimation of firing efficiencies for the replication origins. This approach correctly retrieved origin efficiencies previously determined for chromosome VI and provided testable prediction for other chromosomal origins. Our results gain deeper insights into the temporal coordination of genome duplication, indicating that control of the replication fork rate is required for the timely origin firing during S phase.

1. Introduction

S phase is a critical stage in cell cycle progression where genome duplication has to occur exactly once, without errors, to avoid chromosomal defects that can lead to genomic instability (Machida et al., 2005). This process is realized by firing of multiple chromosomal locations called replication origins (Méchali, 2010), and in the budding yeast genome over 700 potential origin sites have been identified and mapped by genome-wide studies and deep-sequencing technologies (Siow et al., 2012 and references therein). Replication origins are determined and prepared to fire by the binding of initiator proteins that are assembled into pre-replicative complexes, pre-RCs (Bell and Dutta, 2002; DePamphilis, 2005; Sclafani and Holzen, 2007; Takeda and Dutta, 2005), and their phosphorylation by cyclin-dependent kinases (Cdk/cyclin) sets the correct timing of S phase progression (Araki, 2010a,b; Labib, 2010; Sacco et al., 2012; Tanaka and Araki, 2010). Strikingly, Cdk/cyclin complexes synchronize origin firing with cell cycle progression by determining the efficiency of origin firing (Donaldson et al., 1998; Duncker et al., 1999; Katsuno et al., 2009; Krasinska et al., 2008; McCune et al., 2008; Nakanishi et al., 2010; Thomson et al., 2010).

Deregulation (inactivation or alteration in the total amount) of components involved in the control of S phase entrance, such as the pre-replication complex (pre-RC), e.g. proteins forming ORC and MCM complexes, leads to inappropriate firing in the number of origins, resulting in a premature or retarded genome duplication. These abnormalities are the underlying cause of genomic instability and strongly associated to human cancers (Blow and Gillespie, 2008; Hook et al., 2007; Lau et al., 2007; Schär, 2001; Sidorova and Breeden, 2003; Stoeber et al., 2001; Tlsty et al., 1995; Williams and Stoeber, 2012). In budding yeast, genomic stability is compromised when deterioration in the performance of genome duplication

* Corresponding author at: Synthetic Systems Biology and Nuclear Organization, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands. Tel.: +31 20 525 8686; fax: +31 20 525 7934.
E-mail address: M.Barberis@uva.nl (M. Barberis).
[1] Present address: Fritz Haber Institute of the Max Planck Society, Theory Department, Faradayweg 4-6, 14195 Berlin, Germany.

0168-1656/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2013.06.022

or in its surveillance mechanisms occurs (Kolodner et al., 2002). Cdk1 activity plays a role in genome duplication (Enserink et al., 2009): Cdk1/cyclin complexes modulate the replication timing of early and late origin firing (Donaldson et al., 1998; Jackson et al., 2006; McCune et al., 2008; Raghuraman and Brewer, 2010; Schwob and Nasmyth, 1993), and the inhibitor Sic1 (Barberis, 2012a,b and references therein) counteracts their activities to set the kinase threshold for DNA replication, thereby the correct timing of origin firing throughout S phase (Barberis et al., 2007, 2011, 2012; Barberis and Klipp, 2007). In absence of Sic1, DNA replication initiates from few origins, the distance between replicons is 1.5 times longer than wild type and cells accumulate gross chromosomal rearrangement, indication of increased genome instability (Caburet et al., 2002; Lengronne and Schwob, 2002; Nugroho and Mendenhall, 1994). This clearly shows that origin density is crucial for genome integrity and determines S phase dynamics (Bielinsky, 2003), and suggests that either fewer origins could fire with a wild type efficiency, or the firing efficiency could be preserved but the rate of fork progression would decrease.

Replication origins fire at characteristic times (Raghuraman et al., 2001; Yabuki et al., 2002) but exhibit variable frequency of firing in different cell cycles (i.e. efficiency), some firing in almost every cell cycle while others rarely used (Czajkowsky et al., 2008; Friedman et al., 1996, 1997; Raghuraman et al., 2001; Shirahige et al., 1993; Yamashita et al., 1997). As a result, the rate of DNA replication is not uniform throughout the genome, with an average speed of about 3 kb/min (Raghuraman et al., 2001; Rivin and Fangman, 1980; Yabuki et al., 2002). In recent years, deterministic (Barberis et al., 2010a; Brümmer et al., 2010; Gidvani et al., 2012; Spiesser et al., 2009) and stochastic (Barberis et al., 2010b; de Moura et al., 2010; Hyrien and Goldar, 2010; Luo et al., 2010; Retkute et al., 2011, 2012; Yang et al., 2010) models of spatiotemporal activation of replication origins have been developed. Although a few contributions seem to suggest that regulation of origin firing might occur through modulation of the replication fork rate for a timely S phase (Azvolinsky et al., 2009 and references therein), modeling and experimental efforts neglected this aspect by considering the fork speed to be uniform throughout the yeast genome (de Moura et al., 2010; Sekedat et al., 2010; Spiesser et al., 2009; Yang et al., 2010). Indeed, it has been observed that large variations in replication fork rates occur along the yeast genome (Ferguson et al., 1991; Raghuraman et al., 2001; Yabuki et al., 2002), that a significant increase in the fork rate during S phase is observed in mammalian cells (Gauthier et al., 2012; Housman and Huberman, 1975; Painter and Schaefer, 1971; Scott et al., 1997; Takebayashi et al., 2005), and that a gradual and increasing density of fired replication origins occurs in the course of S phase (Farkash-Amar and Simon, 2010; Goldar et al., 2009; Guilbaud et al., 2011; Herrick et al., 2000; Hyrien and Goldar, 2010; Ma et al., 2012; Rhind et al., 2010).

Taking into account this observed variability, a modeling approach representing not homogeneous fork progression rates has been developed to analyse mice cells (Gauthier et al., 2012). Moreover, we have observed that in the budding yeast genome many DNA regions with significant fork rate changes do not show uniform spatial distribution and are clustered on distinct chromosomal locations (Spiesser et al., 2010). It is therefore a challenge to investigate in this organism how fork rate changes may affect efficiency of origin firing for a timely S phase.

2. Materials and methods

2.1. Origins of replication

A total of 206 replication origins identified by the Heavy:Light (HL) timing study (Raghuraman et al., 2001) were considered for the analyses. These origins are annotated in the OriDB database (Siow et al., 2012) with a definite replication timing and correspond univocally to peaks in the replication profiles (Raghuraman et al., 2001). Moreover, 96 valleys which are generated from replication forks departing from two origins traveling in opposite direction were identified.

2.2. Model implementation

A deterministic model of DNA replication was implemented in the programming language Matlab 7.10.0 (The Mathworks Inc.). The input file of the model is specific for each chromosome, and it contains (i) chromosomal location and firing times collected in the OriDB database for each replication origin considered in the study, i.e. only origins with available replication timing determined according to the HL study, and (ii) value of constant or variable fork rate used to reproduce the replication profiles (see below). If the fork rate value is constant, only this value is listed in the input file. If a variable fork rate is implemented, lower and upper boundaries of the range are specified, and an equation is added to ensure a linear dependency between firing times and fork rate. Fork rate progression was then implemented as follows. A local fork rate is assigned to each replication origin and represented by the slope of a line drawn bidirectionally starting from this origin. For all origins considered, the program verifies whether origins that are passively replicated are present along these lines and, if this does occur, their corresponding lines are deleted. The lines terminate only if they meet each other traveling from opposite directions or reach beginning/end of a chromosome. The program stops when the whole length of each chromosome is replicated.

2.3. Model parameters

From the experimental replication profiles (Raghuraman et al., 2001), the following parameters were derived or computed: length of chromosomes, duration of replication, origin density and percentage of symmetric peaks (Table 1). The duration of replication is the timing intercurring between the replication of first and last base pairs in a given chromosome, whereas origin density is calculated by dividing the number of replication origins by the length of each chromosome. In this calculation, only distinct origins, i.e. with a distance between them of at least 2.5 kb, were considered. The percentage of symmetric peaks was calculated from the values of fork rates traveling in opposite directions (see below). Peaks were considered to be symmetric if fork rate values showed a difference <25%. Regions of low density (Raghuraman et al., 2001) were not included in the calculation.

Replication profiles obtained from raw data together with the smoothed curves obtained transforming raw data by using the technique of Fourier convolution smoothing (FCS) (Raghuraman et al., 2001, Supplementary material, part II: Secondary Data Analysis) have been compared (see Supplementary Fig. 1). The smoothening does not change the overall dynamic of the replication profiles, thus resulting in a not biased data analysis.

2.4. Fork rate determination

To calculate fork rate values, experimental replication profiles were scanned to identify peaks (defined as chromosomal regions with a minimum replication timing that increases bidirectionally for a length of at least 7.5 kb) and valleys (defined as chromosomal regions with a maximum replication timing that decreases bidirectionally for a length of at least 7.5 kb). Fork rate values were calculated from the slopes of 2.5 kb fragments departing from peaks and valleys, excluding the near-to-minimum (closer than 2.5 kb) and near-to-maximum (closer than 2.5 kb) regions to avoid errors of smoothing the curves. Only peaks that identify univocally known