Double Rolling Circle Replication (DRCR): Involvement in Gene Amplification and Genome Replication including HSV

Takashi Horiuchi
Department of Molecular Life Science
Division of Basic Medical Science and Molecular Medicine
Tokai University, Kanagawa Japan

Taka-aki Watanabe
Department of Molecular Genetics
Cleveland Clinic Lerner Research Institute, Cleveland, USA
1 Introduction

The phenomenon known as ‘gene amplification’ occurs in many different organisms (Cowell, 1982; Stark and Wahl, 1984; Schimke, 1984; Devonshire and Field, 1991). Much work has been done on gene amplification because of its importance in biology and evolution, and from the practical medical standpoint. However, until very recently, the mechanisms responsible for this process had remained unresolved. In general, there are two types of gene amplification, ribosomal RNA gene (rDNA)-type and oncogene or drug-resistant gene-type. After we had determined the mechanism of rDNA-type gene amplification (Kobayashi and Horiuchi, 1996; Kobayashi et al., 1998; Kobayashi et al., 2001), we addressed the mechanism of oncogene-type amplification. Assuming that a central reaction in oncogene-type amplification was double rolling circle replication (DRCR), we constructed a DRCR-inducing system in yeast and found that two types of amplification products were obtained (Watanabe and Horiuchi, 2005). One product corresponded to HSR (homogeneous staining region)-type products and the other to DM (double-minutes)-type products in cultured cells.

Interestingly, from structural analysis of the HSR-type product, we found that sequences flanked by inverted repeats (IR) were freely inverted. Although this type of free IR recombination is known to occur in HSV-1 DNA (Roizman, 1979; Figure 1(B)), chloroplast DNA (Palmer, 1983; Figure 1(A)) and in 2µ plasmids (Broach and Volkert, 1991; Figure 1(C)), the mechanism(s) remained unresolved. Interestingly, in these cases, the DNA is circular, and has a pair of IR structures when in the host cell. Furthermore, the 2µ plasmid is unique because it replicates in both normal and DRCR mode and is known to be recombinogenic under physiological conditions (Jayaram and Broach, 1983; Figure 1(D)). From these observations, we proposed that DRCR itself markedly activates recombination, as determined using 2µ plasmids. We were able to ascertain that DRCR itself does indeed activate recombination extensively.

When drug-resistant gene amplification is engendered in tissue culture, it is well-established that an initial amplification unit (called an amplicon) has a giant inverted repeated structure. Later, under increasing concentrations of the drug, whereas amplicon gradually shorten, their copy number increases. Although the reason for these changing amplicon processes (called evolution) remained unresolved, we inferred that the following events would be occurring in the initial stage, two pairs of inverted repeats (_____ ← ← ← ← ← ←) are formed spontaneously in the Break-Fusion-Bridge (BFB) cycle, discovered and named by McClintock (1941). We confirmed experimentally that DRCR can initiate from the two pairs of inverted repeats and represents a clear oncogene amplifying mechanism (in preparation). Regarding the shortening and increasing copy number of amplicon during this process, we propose the following. Because there is a large number of repeated sequences, transposable elements and retrotransposons in higher eukaryotes’ (about 50% of the whole genome), highly activated recombination (deletion, inversion and duplication) by DRCR randomly occurs between these repeated sequences under selective conditions. This would result in amplification of advantageous genes, whereas disadvantageous or neutral genes would be lost. This is one aspect of gene evolution. Finally, we propose a model for DRCR-dependent activation of recombination.

2 Gene amplification- Induction of DRCR on Linear Chromosomes

In rDNA amplification, we found that DNA replication fork blocking events at replication fork barriers (RFB) trigger an increase or a decrease in rDNA copy number (Kobayashi and Horiuchi, 1996; Kobayashi et al., 1998; Kobayashi et al., 2001; Kobayashi et al., 2004). On the other hand, for the oncogene
amplification mechanisms studied up to that date, although several models deduced from the experiments using cultured cells had been proposed, they had only been able to partly explain the amplification mechanisms (Hyrien et al., 1988; Ma et al., 1993; Smith et al., 1992; Toledo et al., 1992.). Currently, detailed molecular mechanisms responsible for gene amplification remain to be determined (Debatisse and Malfor, 2005; Tanaka and Yao, 2009; Mondello et al., 2010). Furthermore, although several attempts have been made to artificially induce even partial oncogene-type amplification processes using cultured cells or yeast; these have remained unsuccessful (Ruiz et al., 1988; Butler et al., 1996; Pipiras et al., 1998; Tanaka et al., 2002).

Figure 1: Isomeric structures of chloroplast (cp) DNA, HSV-1 DNA, 2-μm plasmid DNA and two modes (normal and DRCR) of replication in 2-μm plasmids. (A) Two isoform structures of chloroplast (cp)DNA, (B) four isoform structures of HSV-1 DNA, (C), two isoform structures of yeast 2-μm plasmid, (D) two modes of replication of 2-μm plasmids, normal and double rolling circle replication (DRCR).
Our hypothesis concerning oncogene-type amplification mechanisms is as follows: oncogene-type amplification occurs through DRCR (Figures 2A and 5), because it produces products with an inverted repeat (IR) structure. We chose budding yeast as a model system, and tried to induce DRCR using three different recombination systems: (1) break-induced replication (BIR) (Kraus et al., 2001), (2) site-specific Cre-lox recombination (Stark et al., 1992) and (3) homologous recombination. Although DRCR had been proposed as one of several models to explain oncogene-type amplification (Hyrien et al., 1988), the initiation of DRCR is different from ours and to the best of our knowledge, the hypothesis has not yet been put to the test.

![Diagram](image)

**Figure 2:** Recombinational process coupled with replication. (A) DRCR. Two replication forks chase each other. One replication fork moves faster than the other. (B) Recombinational process coupled with replication. The gray and black lines indicate the un-replicated and recently replicated regions at the time of recombination, respectively. If recombination occurs between loxP sites marked red and blue (i), the replication template is switched and thereafter the replicated region is replicated again (ii). (C) DRCR induction. If both bidirectional DNA replications undergo the processes as described in (B), DRCR can be induced.

Although DRCR induction is accomplished by all three methods, here we illustrate how Cre-lox recombination initiates DRCR, as this is the easiest to understand. DRCR in linear genomes is illustrated in Figure 2A. Two replication forks follow each other around a circle of DNA, from which two linear
DNA double strands elongate continuously in opposite directions. In order to induce DRCR, first, as shown in Figure 2B, a pair of inverted lox sequences is arranged at the left side of a replication origin (O). When a replication fork initiated from the replication origin advances left and arrives at the region between a pair of lox, recombination between inverted lox sites changes the direction of the fork (in Figure 2B(i) to (ii)), and one DRCR-type replication fork is formed. If another recombination event occurs rotationally symmetrically to the right of the replication origin (Figure 2C), two replication forks should follow each other and initiate DRCR. To terminate DRCR, two reverse recombinations change the replication forks from following one another to bi-directional forks, although a large number of inverted repeats remain behind.

3 Gene amplification: Two Types of Amplification Depending on Initial Recombination of DRCR

If an alternative type of recombination occurs not rotationally symmetrically, but plane symmetrically (Figure 3, on the right), two replication forks should meet, producing an acentric circular genome with a pair of large IR structures, with retention of the original linear genome. Thus, in a linear genome, DRCR is expected to produce the two kinds of products shown in Figure 3, one being an HSR-type product (the linear genome with a large number of inverted repeats) and the other DM-type products (the acentric multi-copy circular mini-chromosome with a pair of inverted structures). In fact, we carried out DRCR gene amplification experiments using the Cre-lox system (Stark et al., 1992) in yeast and did indeed obtain two kinds of products: one being a chromosome with a large number of genes in inverted repeats, and the other a high copy number of linear not circular mini-chromosomes with an inverted structure. The reason why we obtained linear but not circular mini-chromosomes is because we inserted the lox sequences into the terminus region of chromosome VI (Watanabe and Horiuchi, 2005; Watanabe et al., 2011).

When DRCR initiates or terminates, if recombination occurs asymmetrically at initiation, a chromosome with a giant IR structure should be produced and at termination, a similar chromosome with a giant IR structure should be produced, but at the center of the chromosome, amplified region should be inserted.

What is the mechanism for gene amplification under natural conditions? Around 1990, several groups (Ruiz and Wahl, 1988; Smith et al., 1992; Ma et al., 1993) reported that the initial step of gene amplification can occur via the BFB cycle, a process first described by McClintock (1941). She reported that double strand breaks occur at a site into which a transposable element is inserted, and this initiates the BFB cycle. In addition, we speculate that TS (temperate switching; see Figure 4A) is another trigger for the BFB cycle. Our artificial chromosomal structures are shown in Fig. 4B (one is the Cre-lox system and the other consists of the two pairs of inverted structures). Figures 4C and 4D show that two types of gene amplification, DRCR and CR (convergent replication) produce HSR type and DMs products, respectively.
4 Extreme of DRCR and its Consequences: Evolution of Amplicon under DRCR

A key question remains. From the microscopic observation of the process of gene amplification in cultured cells, as we have seen above, the amplification unit (amplicon) gradually shortens and the copy number increases with increasing drug concentration. This phenomenon is called ‘evolution’. Such evolutionary changes remain to be accounted for. We may regard this phenomenon as gene evolution, because the cell possesses mechanisms to select and amplify genes that are advantageous for the cell itself. How does such a complex change come about? A key to solving the mystery was found in the first successful experiment, in which gene amplification was achieved by DRCR initiated by the BIR method (Watanabe and Horiuchi, 2005). When we analyzed the structure of amplified HSR-type products with restriction enzymes, we found that, in addition to the products with the expected structure, those with an unexpected structure also emerged. To our surprise, the ratio of expected to unexpected products was 1:1.
Figure 4: Initiation mechanism of DRCR under natural conditions; Involvement of BFB (Break-Fusion-Bridge) cycle A. Template switching (TS); When DNA replication proceeds to a center of a pair of IR structure, recombination occurs between the pair of IR and the direction of the replication fork is changed as shown in Figure 4A, producing a di-centromeric chromosome. Thus, in mitosis, chromosomal breaking occurs, after replication of the breaking sister-chromatid, the two sister-chromatids are fused, producing a dicentromeric chromosome. Because this chromosome structure is corresponding to structure of four lox sites, shown in B, and thus this (Double inverted repeat; double IR) is a starting structure of DRCR. From this structure, two alternate processes initiates; C is DRCR, producing HSR structure, or D is circle replication, producing a circle molecule with IR structure.
If DCR proceeds as expected, the structure of the products should be as shown in the upper line in Figure 5, with two major bands of 4.4 kb and 10.3 kb produced by XhoI digestion. However, the observed gel pattern was unexpected, in that two other dense bands of 8.2 kb and 6.4 kb were produced, as shown on the left of Figure 5. This pattern can only be explained by assuming that a pair of fixed IR leu2d gene structures is freely inverted. The amounts of these unexpected fragments were so large that all four DNA bands could be detected not only by southern hybridization, but also by EtBr (ethidium bromide) staining. The same results were obtained not only for DCR induced by Cre-\textit{lox}, but also homologous recombination, thereby strongly suggesting that the DCR process itself may be recombinogenic. To test this hypothesis more rigorously, we investigated recombinogenic properties using the 2\mu plasmid system.

![Diagram of DCR](image)

**Figure 5:** DCR-dependent free inversion. DRCR is expected to produces the product whose structure is shown in the upper line and its XhoI digestion products is expected to be two main DNA fragments, 10.3 kb and 4.4 kb in length. However, the actual digestion pattern, shown in left side, shows four DNA fragments, 8.2 kb and 6.4 kb in addition to 10.3 kb and 4.4 kb. Thus, these results indicate that free inversion occurs between red regions occurs.

The 2\mu plasmid is the only known example of a replicon which replicates in DRCR mode under natural conditions. It is a double stranded circular DNA (~ 6.3 kb) found in budding yeast. Interestingly, it can be maintained by normal replication, but depending on circumstances, for example, when the copy number is decreased, it replicates in DRCR mode to recover copy number quickly (Volkert and Broach, 1986). The reason that the 2\mu plasmid can replicate by DCR is that it has a site-specific recombination system, like Cre-\textit{lox} (Broach, \textit{et al}., 1982). The plasmid has a pair of IR (0.6 kb), where a site-specific recombinational sequence (\textit{FRT} sequence) is located and a site-specific recombinase (Flp protein) is encoded on the plasmid. Thus, as shown in Figure 1, normal replication starts bi-directionally from the rep-
lication origin, but a recombination occurs between two FRT sites mediated by the Flp protein, and the replication mode changes from bi-directional replication forks to those following one another. This recombination initiates DRCR and the plasmid copy number increases rapidly (Volkert & Broach, 1986). This DRCR ability of the 2µ plasmid was first deduced theoretically from the genome structure by Futcher (1986) and soon afterwards Broach’s group confirmed experimentally that the plasmid can replicate in two replication modes, one normal and the other DRCR. Under natural conditions, two structural isomers are present in equal amounts. Thus, it is to be expected that other plasmids or virus DNAs exist, with a pair of IR structures, like the 2µ plasmid DNA, and which could also replicate in DRCR mode. Furthermore, if the length of the IR is substantial, homologous recombination should initiate DRCR.

Interestingly, Broach’s group previously reported that recombination was enhanced in 2µ plasmids (Jayaram and Broach, 1983). Therefore, we postulated that DRCR itself is recombinogenic and we repeated their experiments. We used 2µ plasmid DNA into which transposon Tn5 (IR) was transposed. Tn5 (IR) consists of three parts, the center region containing a kanamycin-resistance gene, and two inverted arms. We then introduced the Tn5-transposed 2µ plasmid DNA into yeast, cultured them, and then extracted DNA. We reconfirmed Broach’s results that Tn5 is frequently inverted, but in the site-specific FLP recombinase-defective plasmid, it could only undergo normal replication and Tn5 (IR) inversion was not observed. Next, we changed the structure of Tn5 from a form with the arms inverted to directly repeated arms [Tn5 (DR)]. We found that in the 2µ plasmid with the Tn5 (DR), duplication and deletion of directly repeated arms occurs frequently. However, no structural changes were seen in the site-specific recombination-defective plasmid. Finally, we inserted Tn5 (DR) into the DRCR amplifying unit of the yeast chromosome, induced DRCR and examined the structure of the amplified products. In the amplified region, we found that deletion and duplication of Tn5 (DR) occurred frequently. From these results, we concluded that, regardless of whether the genome was linear or circular, DRCR itself is highly recombinogenic (Okamoto et al., 2011).

So how is DRCR recombinogenic? Actually, when DRCR was induced in yeast, recombination is saturated, that is, the amount of substrate for recombination equaled the amount of recombination product. We propose a model, in Figure 6, which explains how DRCR becomes so recombinogenic. Normal replication proceeds simultaneously with the production of a pair of sister chromatids, held together by a protein complex, “cohesin”. However, unlike in normal replication, as shown in Figure 6, in either circular or linear genomes, the DRCR replication fork is expected to forcibly separate the chromatids, thus freeing them from one another, resulting in what we termed ‘a single daughter chromatid’, which is expected to be recombinogenic. This model is supported by two studies, one of which was by us (Grossenbacher-Grunder and Thuriaux, 1981; Kobayashi et al., 2004). Both reported that homologous recombination is enhanced in mutants partially defective in cohesion. If this model is correct, cohesin should have an additional function as an anti-recombinogenic.

To return to our question, what is the relationship between recombinogenic DRCR and evolution of am- plicon, we suggest that there are two mechanisms responsible for the recombinogenic properties of DRCR. One is the disarrangement of regularly repeated sequences. Regularly repeated sequences should render the amplified region unstable, but randomization of the orientation of inverted repeats by DRCR stabilizes the amplified region. The second is that, in the higher eukaryote genome, there are a very large
number of repeated sequences, including transposons, insertion sequences, retro-transposons, etc. The total amount of these repeated sequences is estimated at about 50% of the entire human genome. If recombinogenic DRCR acts on such chromosomes containing these repeated sequences, a large number of recombination events, including deletion, inversion, and duplication, should often occur between repeated sequences, during the amplification of different regions. As a result, under conditions selecting for drug resistance, only clones with a large number of amplified resistance genes will survive. In contrast, clones with amplified unnecessary or disadvantageous regions or genes will be eliminated. This is the evolution of gene amplification. Interestingly, in our gene amplification experiments using yeast, this kind of evolution of gene amplification in cultured cells has not been observed. This can be explained by the very low amount of repeated sequences (about 2%) in the yeast genome relative to the higher eukaryote genome.

To return to our question, what is the relationship between recombinogenic DRCR and evolution of amplicon, we suggest that there are two mechanisms responsible for the recombinogenic properties of DRCR. One is the disarrangement of regularly repeated sequences. Regularly repeated sequences should render the amplified region unstable, but randomization of the orientation of inverted repeats by DRCR stabilizes the amplified region. The second is that, in the higher eukaryote genome, there are a very large number of repeated sequences, including transposons, insertion sequences, retro-transposons, etc. The total amount of these repeated sequences is estimated at about 50% of the entire human genome. If recombinogenic DRCR acts on such chromosomes containing these repeated sequences, a large number of recombination events, including deletion, inversion, and duplication, should often occur between repeated sequences, during the amplification of different regions. As a result, under conditions selecting for drug resistance, only clones with a large number of amplified resistance genes will survive. In contrast, clones with amplified unnecessary or disadvantageous regions or genes will be eliminated. This is the evolution of gene amplification. Interestingly, in our gene amplification experiments using yeast, this kind of evolution of gene amplification in cultured cells has not been observed. This can be explained by the very low amount of repeated sequences (about 2%) in the yeast genome relative to the higher eukaryote genome.
5 Involvement of DRCR in Natural Genome Replication: A Model for HSV-1 and Chloroplasts

As described above, because the 2µ plasmid DNA is replicated (amplified) by DRCR, it would not be surprising if other plasmids or viruses also replicated their DNA by DRCR. Even today, there remain many plasmids and virus whose DNA replication modes are still unknown. Here, we consider Herpes Simplex Virus (HSV) and chloroplast DNA. Their DNAs are both double stranded, but the former is linear and the latter circular. Chloroplast and 2µ plasmid DNA are structurally very similar in that both contain a pair of IR structures (Tobacco chloroplast 25.3kb each IR/156 kb total length (Kolodner and Tewari, 1979); 2µ 0.6 kb each IR/6.3 kb total length (Broach and Volkert, 1991)) and both consist of equal amounts of two structural isomers, suggesting frequent inversion occurring between IRs. Moreover, several mitochondrial DNAs (mtDNA) also have this type of structure (for example, mtDNA of Achlya (Hudspheth et al., 1983)). Furthermore, when HSV-1 infects cultured cells, its DNA is immediately circularized, which makes its structure very similar to chloroplast and the 2µ plasmid DNA (15.5kb each IR/152kb total HSV-1 length). Their replication intermediates possess a large number of branched structures and are very complicated (Zhang et al., 1994; Severini et al., 1996). For example, even if HSV-1 replication intermediates are treated by a restriction enzyme, which can digest one cut per single HSV-1 DNA unit, structural analysis using PFGE or Field Inversion techniques reveals that the majority of the sample DNA remains in the original well (Severini et al., 1994; Zhang et al., 1994). During DNA replication, homologous recombination between two IRs is expected to occur so frequently that two or four structural isomers are produced at a 1:1 ratio in chloroplasts and 1:1:1:1 in HSV, suggesting a significant association between replication and recombination (Roizman, 1979; Bataille and Epstein, 1995; Umene, 1999; Wilkinson and Weller, 2003). DRCR, which is a highly recombinogenic process, accounts for all these properties.

However, there are several exceptions to this rule for both chloroplast DNA and HSV. For example, chloroplast DNA in allied species in the subfamily Papilonoidea of the legume family (Fabaceae) and Douglas-fir and radiata pine do not have an IR structure but rather a single rDNA gene. Thus, they must be unable to replicate by DRCR. However, Bendich reported that replication intermediate of pea chloroplast DNA with only one rDNA has a complex structure like chloroplast DNAs with IR (Bendich 2004; Bendich 1991). The replication intermediate structure is currently still unresolved. A likely explanation is that they may replicate in the same manner as mtDNA type in organisms that are not animals, but this mechanism remains to be determined.

In HSV-1, it has been well established that replication is associated with recombination. We mentioned above that an equal number of viruses with four different structural isomers are produced by recombination associated with replication. Furthermore, when Tn5-transposed HSV-1 DNA replicated it inverted at high frequency, in the same manner as Tn5-transposed 2µ plasmid (Weber et al., 1988). These observations can be explained well by DRCR–dependent recombination. Here, we have presented a DRCR-dependent HSV-1 replication model. Previously, we proposed the model illustrated in Figure 7-1 (Okamoto et al., 2011) on the basis of the report that after HSV-1 infection, the viral DNA does not circularize, but remains linear (Jackson & DeLuca, 2003). However, a later paper from a different group reported findings completely opposite from those of Jackson & DeLuca (Strang & Stow, 2005). Taking this into account, here we present two models, one of which is a linear model (Figure 7-1) and the other circular (Figure7-2). In the latter model, DNA is contiguous and some replicates normally, while some does so in DRCR mode.
Figure 7 (I): HSV-1 DNA replication model (Linear model). Red lines indicate single daughter chromatid.
Figure 7 (2): HSV-1 DNA replication model (Circular model). Red lines indicate single daughter chromatid.

On the other hand, it has been generally thought that rolling circle replication (RCR) is involved in DNA replication of HSV-1 (Roizman, 1979; Lehman and Boehmer, 1999; Weller and Coen, 2012). However, as described above, replication intermediates have highly complex structure with a large number of DNA branches. HSV-1 DNA has three replication origins (one OriL and two OriS), to which the viral coded protein, UL9 (or OBP), binds (Elias et al., 1986) and initiates DNA replication. By using the temperature sensitive UL9 mutant viruses, UL9-dependent DNA replication was found to be limited to the first 6 hours after infection. Subsequently, UL9-independent replication occurs and the huge mass of viral DNA accumulates (Blümel and Matz, 1995; Schildgen et al., 2005). These results suggest that HSV-1 replication is not so simple like RCR, but more complex and recombinogenic, producing equal number of four types of genomic isomers. Thus, so far, there have been several reports alluding to the possibility that replication of HSV DNA might be by DRCR. However, they did not refer to potential recombinogenic properties of DRCR (Hammerschmidt and Mankertz, 1991; Zhang et al., 1994; Grossenbacher-Grunder and Thuriaux, 1981, Severini et al., 1996).

There is another piece of evidence that HSV-1 replicates in DRCR mode. The herpes virus family contains a large number of members, some with a DNA structure quite different from HSV-1. One of them, HHV-6, has DNA with only a 10 kb DR structure at two terminus ends; after infection, virus DNA is thought to be circularized but lacks any other homologous regions required for DRCR. Investigations on HHV-6 replication intermediates after virus infection showed that DNA was circularized but its replication intermediates were only simple linear-multimer, circular-monomer, and -oligomer forms, with no branched structures (Severini et al., 2003). These data strongly suggest that HHV-6 replication is not by DRCR, but solely normal.

EHV (Equine Herpes Virus) and VZV (Varicella Zoster Virus) are further members of the herpesviridae, but although their DNA structures are similar to HSV-1, they have only two or four structural isomers. However, when there are four isomers two are major and the other two minor. After infection, if these genomes are circularized and replicate in DRCR mode, such unbalanced isomer formation could
not be explained. However, further subsequent reports revealed that in the replication step of virus DNA, although equal amounts of the four structural isomers are present, in the packaging step, different distributions of major and minor virus DNA isomers appear. This suggests that the unbalance is not relevant for DNA replication (Slobedman and Simmons, 1997; Schynts et al., 2003).

In HSV-1 and closely related viruses, several mutants have been isolated in which a common region between UL and Us is deleted. According to our model, these mutants would be predicted to be unable to replicate in DRCR mode. Unexpectedly, however, when these viruses infect cultured cells, there is a slight drop in productivity, but growth is little affected (Sauer et al., 2010). However, similar experiments using a mutant Equine Herpes Virus (which is closely-related to HSV-1) with a similar common region deletion resulted in a smaller plaque size and delayed growth kinetics. On the other hand, when animals infected directly with these viruses, growth rates of the mutants were significantly lower than seen for wild-type viruses, and pathogenicity was also decreased (Jenkins and Roizman, 1986; Poffenberger and Roizman, 1985; Poffenberger et al., 1983). These results suggest that DRCR might play an important role in vivo, but in vitro HHV-6 type replication described above might occur.

6 Conclusion

Until now there are some amplification whose mechanisms have yet to be elucidated. One is the gene amplification occurred in H region of genome in *Leishmania* protozoa (Olmo et al., 1995) and the other is ‘palindrome’ type amplification of petite (rho) mutants of budding yeast (Rayko and Goursot, 1996). The formers are two types, circle and linear, circle type can be produced by DMs type reaction (see DM production in Figure 3), linear type can be done by giant palindrome formation (see page 9, lines 2-4). The latter type of amplification unit coincides with that of DRCR amplification unit. Thus, rho palindrome type amplification is very likely to be produced by DRCR. The characteristic of this inverted repeat is short unit (1~2kb).

DRCR has long been believed to be an exceptional DNA synthetic mechanism unique to the yeast 2µ plasmid. As we discussed in this review, however, DRCR have more extensive roles in other biological systems. We predicted and subsequently found, for example, that DRCR is involved in oncogene-type gene amplification. Importantly, DRCR appears to be intrinsically recombinogenic, and this characteristic must be important in stabilizing repeated structure of amplified products in lower eukaryotes, and in “evolution” of highly repeated short amplicon from a small number of long amplicon in higher eukaryotes. In a fashion similar to our model system, DRCR may have been involved in natural gene evolution.

In addition to well-documented DRCR involvement in 2 micron replication, we speculate that DRCR is also important in replication of other natural replicons such as chloroplasts, mitochondria and HSV. In the case of mitochondria, many types of replicons, some circular and others linear have been documented. In this case, DRCR involvement may not be universal. In contrast, most if not all chloroplast DNA has a pair of inverted repeat structure. It is therefore possible that chloroplast DNA is exclusively replicated through DRCR because of yet unknown advantages in chloroplast function.

We described that combination of amplification and recombination, expected for DRCR, can explain extreme complexity of HSV-1 DNA structure. It should be noted that amplified products here also contain replication origins, from which further replication can initiate. Explosive increase in viral DNA and particles could be the result of these processes, which are quite different from more classical and extensively studied cellular genome replication.
HSR-type DRCR amplification produces highly repeated sequences. It has been generally postulated that multiplication of genes has a major role in evolution partly because it generates functional redundancy. In the case of Neurospora crassa, a phenomenon referred to as RIP (repeat-induced point mutation) is documented (Galagan and Selker, 2004). RIP, which is induced only after fertilization but before DNA synthesis or nuclear fusion, results in extremely abundant mutations restricted on repeated DNA. By combining DRCR with RIP, we imagine that it may be possible to device a bio machine for artificial gene evolution, in which highly repeated and rapidly mutated genes can evolve rapidly. Furthermore, RIP may not be unique to Neurospora crassa; mutations may be induced on repeated DNA in other organisms also, although may not be as extensive as in Neurospora crassa. If that is the case, repeated genes, such as those generated though DRCR, could have been important in evolution not only because the functional redundancy but also because of their high mutation rates.

Acknowledgement

In writing this review, we thank a lot of members for helping and encouraging us. Especially, we would like to thank Dr. Haruko Oksmoto (Toyota Central R & D Labs. Inc.) for carrying out experimental work on recombinogenic property of DRCR, Dr. A. J. Bendich (Univ. Washington) for giving us information of replication of chloroplast DNA and Dr. K. Umene (Fukuoka Woman’s University) for discussing about HSV DNA replication. Furthermore, we give a special thanks Dr. M. Tanaka (Tokai Univ.) for critically reading the manuscript and giving invaluable advice, Dr. M. Kimura (Tokai Univ.) and Dr. H. Inoko (Tokai Univ.) for continuous supporting and encouraging us.

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