# C. Genetics

## I. Replication

By Walter Nagl

### 1. Introduction

It is now well established that particular interphase chromosomes have specific domains of residence and positions with respect to each other in the interphase nucleus, thus occupying "chromosome domains" (reviewed by Hilliker and Appels 1989; Manuelidis 1990; Haaf and Schmid 1991, and for plants, Heslop-Harrison and Bennett 1990). The chromatin itself is organized by nonhistone proteins into discrete entities, visible (by increasing order of size and level of condensation) as loops, rosettes, chromatin domains, chromomeres, and chromocenters (Reznik et al. 1991). The resulting large-scale and fine-scale nuclear structures are evidently obtained by attachment of certain DNA sequences on the nuclear matrix (or scaffold) and the nuclear envelope, particularly the pore complexes. There is growing evidence indicating that nuclear structure is involved in the regulation of DNA synthesis (Laskey et al. 1989).

The emphasis of this report will be seen in the relationship of molecular and nuclear structure in the control of DNA replication and the cell cycle in all, as well as in differential replication (e.g., amplification). New results on the origins, the initiation, and the enzymes of replication will be also briefly discussed. I should like to stress that only selected references are given out of the huge amount of new publications, which usually mainly deal with animal systems.

### 2. Nuclear Structure and DNA Replication

The organization of eukaryotic DNA and chromatin into topologically independent loops and domains by attachment of specific sites to a nonhistone nuclear skeleton is now generally accepted. This proteinaceous structure that remained after membrane and histone extraction was variously called nuclear scaffold, matrix, cage, skeleton, and even different (Fig. 1; for a review see Gasser et al. 1989).

Findings in several systems suggest that scaffold (matrix) attachment regions (SARs, MARs) may be loci of putative origins of replication. SARs are AT-rich and contain recognizable motifs, they are found exclusively in noncoding sequences, and they are often a component of an autonomously replicating sequence (ARS). It seems that the association with the scaffold is a feature of replication origins conserved from yeast to humans (Gasser et al. 1989; Phi-Van and Strätling 1990). Actually, not only initiation is proposed

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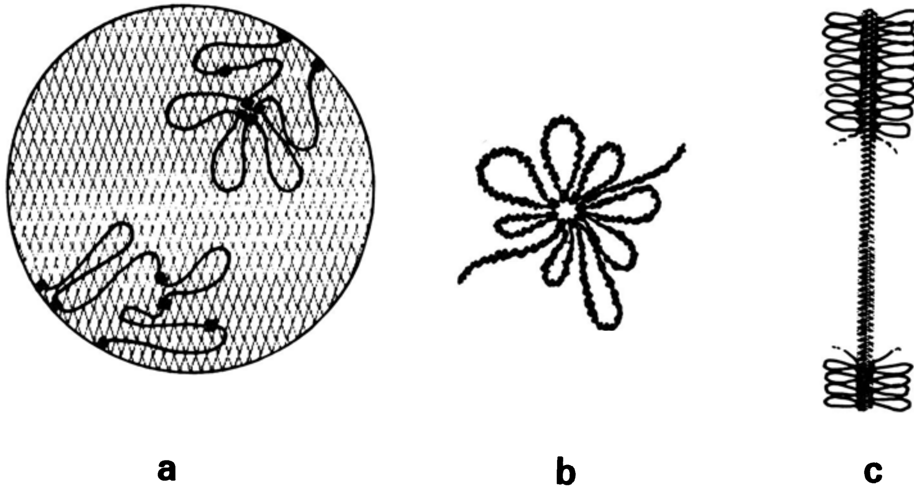



**Fig. 1.** **a** Schematic view of the nuclear location and reorganization of chromatin loops and domains during the cell cylce (not to scale). *Squares* indicate matrix associated regions (MARs) that are attached to the nuclear matrix and the nuclear envelope. **b** In many cases several MARs associate to form a domain, or even a chromomere. Dependent on the availability of origins, the DNA of a single loop or of a chromomere may be active as one replicon. **c** During mitosis the nuclear matrix and the MARs are respectively reorganized and polymerized to the chromosome skeleton (the loops then can only be visualized after extraction of histones)

to occur at the nuclear scaffold, but it has also been widely presumed that the replication complex itself is matrix-bound, and that the topologically constrained, looped domains in nuclei are identical with replicons ("replicon domains"). Vaughn et al. (1990a) reported that the matrix-attached DNA fraction was found to be markedly enriched for replication forks.

Opstelten et al. (1989) isolated and sequenced short direct repeats at nuclear matrix-associated DNA regions from *Physarum*, and have shown that they possess the potential of playing a crucial role in the control of a single replication and of the entire genome in the cell cycle.

The other nuclear structure involved in replication appears to be the envelope with its pore complexes. Blow and Laskey (1988) have shown that membrane integrity is essential for properly limiting DNA replication to a single round per cell cycle, and Leno and Laskey (1991) found evidence from studies on *Xenopus* egg extracts that even the timing of replication is determined by the nuclear membrane.

## 3. Cell Cycle and S Period

DNA replication in eukaryotes is restricted to the DNA synthesis (S) period of the cell cycle. The control of that phase can be seen on two levels, the biochemical and the structural. The biochemical one involves recognition of origins, denaturation of the duplex, priming the template, synthesis of daughter strands, removal of primers and ligation

of new fragments (see Nagl 1989). The structural side involves a series of events extending from the molecular to the light microscopic level. At the molecular level, replication might pass through nucleosomes without histone displacement (Bonne-Andrea et al. 1990). This indicates that the histone octamers remain associated with the mother strand, and that intact nucleosomes re-form on the daughter helices. There is now good evidence for a preferential sequestering of histones onto replicated DNA, that DNA replication promotes chromatin assembly and facilitates precise folding of DNA in the nucleosome (e.g., Almouzni and Méchali 1988; Almouzni et al. 1990; Gruss et al. 1990). DNA topoisomerases I and II activities seem to be essential for DNA replication, but not for chromatin assembly (Annunziato 1989; Gedik and Collins 1990). In contrast to this assumption, the assembly of nucleosome-like structures is mediated by cauliflower DNA topoisomerase in an in vitro system including pBR322 DNA and cauliflower histones (Fukata et al. 1989).

At the higher structural levels, replication (and it should be noted, also transcription) is preceded by decondensation of chromatin. Actually, chromatin is dispersed progressively during cell traverse through the cell cycle (an idea originally put forward by Mazia 1963), particularly through the G1 phase, to reach its maximum dispersion in early S, and then recondensed.

Recondensation that follows DNA replication proceeds faster than decondensation in the S phase. In consequence, the well-known patterns of replication are found, as different domains replicate during discrete intervals of the S phase (see Nagl 1979, 1989). Takahashi (1989) developed a model of chromatin-dependent DNA replication sequences on his previously reported "decondensation units" hypothesis (Takahashi 1987).

The sequence of replication of domains is well reflected by the relative replication time of every Giemsa G-band in animal and human cells, and this sequence is apparently as conserved during phylogeny as the banding pattern and karyotype structure (e.g., Stanyon et al. 1990). This result can again be seen from a biochemical and a structural point of view. Both aspects are related to DNA methylation, which is now generally assumed to act as a condensation mechanism and cell memory in gene silencing (epigenetic imprinting theory; Cedar 1988; Riggs 1989). However, the relationship to replication is not yet clear. Boye and Løbner-Olesen (1990) reached the conclusion that methylation is required for initiation. Their findings, although obtained in *E. coli*, possibly explain underreplication and amplification in eukaryotic cells. At lower levels of methyltransferase, some of the origins may be not methylated, and hence not initiated; at higher enzyme levels, some origins may be methylated too fast and hence be initiated twice (or more) within the same S period or replication window. Contrarily, there is good evidence from many eukaryotic (both animal and plant) systems, that methylation is connected with chromatin condensation, gene inactivation, late replication and even noninitiation (Riggs 1990; see also Poot et al. 1990).

It should be called to mind that just a few selected aspects are dealt with in this chapter. The interesting progress in the elucidation of cell division cycle (CDC) genes (e.g., Gatti and Baker 1989), the universal maturation promoting factor (MPF), as well as the phosphorylation/dephosphorylation of several factors, which are also involved in the controlled sequence of complete DNA replication and mitosis (for a review see Hartwell and Weinert 1989), are outside the scope of this review. Also replication-transcription

coupling and cell type expression, and even cellular life span must be recalled in this context.

## 4. Origins and Initiation of DNA Replication

Studies on DNA replication and its regulation in eukaryotic cells have been limited in part by the lack of useful genetic approaches, but also by the feeling that the mechanism of DNA replication in eukaryotic cells will merely reflect what we have already learned from studying bacteria and their phages. This view ignores the unique problems specific to eukaryotes and, perhaps incorrectly, assumes that mechanisms will be the same (Stillman 1989). Linskens and Huberman (1990) showed that the conflicting data on the size of eukaryotic replication origins, i.e., either a broad initiation zone (Vaughn et al. 1990b), or an origin less than 450 nucleotides in length (Burhans et al. 1990), are not necessarily reconcilable. Instead, they suggested that the two sets of data may illuminate two different faces of chromosomal origins, as they result from the technique employed and the resolution obtained (Fig. 2). The shown model assumes that OBRs (origins of bidirectional replication) and ori's (origins of replication) are not identical.

Many studies were made on autonomously replicating sequences (ARS), as originally isolated from yeast (see Nagl 1989). It is now well established that ARS own a highly conserved sequence (e.g., Grodberg et al. 1990; Sudo et al. 1990; van Houten and Newlon 1990) and structure, i.e., a specific bent form, in bacteria (Eckdahl and Anderson 1990) like in yeast, in plants (Eckdahl et al. 1989) like in humans (Valenzuela 1990). Besides of several proteins binding to ARS and ori sequences, the thermodynamics of helix unwinding (Umek and Kowalski 1990) and the conformation of DNA in the vicinity of ori's, such as left-handed Z-DNA and triplex structures (Bianchi et al. 1990) were studied. However, all the excellent model studies do not contribute to the general structure and function of origins in living eukaryotic cells so far, as there are evidently additional factors controlling replication. For instance, many ARS elements are active when located

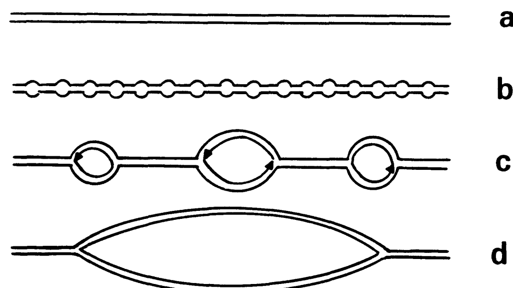



**Fig. 2 a–d.** Diagram to show the size of initiation zones of DNA replication as detected by the employment of different methods. **a** An origin of bidirectional replication (OBR) as found by sequencing. **b** Abundant microbubbles as visualized by electron microscopy. **c** Minibubbles (bidirectional and unidirectional replication forks) as detected by 2 D gel electrophoresis. **d** Macrobubble, the characteristic initiation zone of a replicon (10–30 kilobases) as observed in studies using light microscopic fiber autoradiography. (After Linskens and Huberman 1990)

in plasmids, but not in their normal chromosomal environment (Huberman 1990). Possibly, this behavior is the consequence of a position effect exerted by the telomere. The number of replicons initiated in plants cells was studied by the aid of fiber autoradiography. With regard to systematic, i.e., evolutionary differences, Olszewska et al. (1990) studied replicon sizes and fork migration rates in six diploid annual species with different 2C DNA values and S phase durations, and suggested a relationship between DNA chain elongation and the amount of repetitive sequences. Concerning ontogenetic differences, Houssa et al. (1990) observed halving of replicon size by cytokinin application to the shoot apical meristem of *Sinapis*, and hypothesized that one of the proteins involved in the initiation of DNA replication is a target for cytokinins. Most of the studies were made in animals, where regulation of replicons in different cell types varies drastically both at the level of availability of origins and the time of initiation during S phase. Different models for the regulation have been proposed (e.g., Diffley and Stillman 1990), but the pattern of chromatin condensation apparently plays an important role.

With the recent progress in sequencing DNA, the data obtained indicate that signal sequences for replication and transcription are closely related. As for a recent example, Ariga et al. (1989) showed that the oncogenic c-*myc* protein-binding sequences are indispensable for both replication ori and transcriptional enhancer functions, but that additional sequences are required for maximal ori and enhancer activities. Thus, the same protein can be a sequence-specific factor which is apparently used both in initiation of DNA replication and in regulation of RNA transcription (see also Iguchi-Ariga and Ariga 1989; van der Vliet 1989). In the last few years, there has been notable progress in understanding the basis of the immense specificity for the localization and control of DNA transactions as replication, transcription, and recombination. The unifying principle is that DNA-bound proteins interact to generate a multiprotein regulatory complex in which the intervening DNA is looped or wound (reviewed by Echols 1990). With this we reach again chromatin structure as described at the beginning of the present chapter.

## 5. Enzymes and Proteins of Replication

Although DNA replication of chromosomes possesses unique features, the fundamental mechanisms are common to both prokaryotes and eukaryotes, and the biochemistry of initiator proteins, the structure of the replication fork, and the regulation of initiation show striking evolutionary conservation (Marraccino et al. 1990). A valuable tool in the study of cellular DNA replication has been proven to be the SV40 genome, which contains a single origin and is organized into a minichromosome containing histones (nucleosomes) derived from the host cell. With the exception of the virus-encoded large T antigen, all the proteins required for SV40 replication are supplied by the host cell. Thus, many of the essential host proteins probably play a similar role in cellular DNA replication. The in vitro SV40 DNA replication system has been established by Li and Kelly (1984), and since this time much information on eukaryotic DNA replication was derived from this model system. Reviews have been published by Burgers (1989), Challberg and Kelly (1989), Stillman (1989), Hurwitz et al. (1990), Richardson and Lehman (1990), and Thömmes and Hübscher (1990a).

Only in recent years have we become aware of the fact that DNA replication is mediated by a large multienzyme complex that can quite reasonably be viewed as a true "replication machine"; a sophisticated DNA-handling apparatus, whose numerous protein parts move relative to each other, without disassembling as the replication fork moves. In retrospect, it seems obvious that complex living systems could not exist without the "high technology" of such multicomponent protein machines, that cause the ordered conformational changes in protein molecules by the hydrolysis of bound nucleotidtriphosphate molecules (Alberts 1985).

Some basic rules of DNA replication valid for all organisms are the following (according to Thömmes and Hübscher 1990a):

1. Sequence-specific DNA binding proteins recognize and possibly open specific DNA sequences (ori's).
2. Strand separation is achieved by enzymes called DNA helicases.
3. Chain initiation is performed through RNA priming by an primase.
4. DNA polymerases hydrolyze deoxyribonucleotide 5'-triphosphates adding dN-monophosphates to the growing chain and releasing pyrophosphate.
5. Polymerases are guided by a template via the base-pairing rule.
6. The direction of polymerization is 5'->3'.
7. Various auxiliary proteins are required for priming, efficient primer recognition, processivity, fidelity, coordination, and recycling. This results in different subassemblies for leading and lagging strand synthesis.
8. Further enzymes involved are ribonuclease H, 5'->3' exonuclease, ligase, 3'->5' exonuclease.
9. Release of topological stress is achieved by DNA topoisomerases I and II.

### a) Helicases

There are at least ten enzymes capable of unwinding duplex nucleic acids in *E. coli*, and evidently also in eukaryotic cells, and each enzyme appears to have a specialized role in DNA replication, but also in scanning pre-mRNA for splicing signals and mRNA for translation initiation codons (reviewed by Matson and Kaiser-Rogers 1990; Thömmes and Hübscher 1990b). Gorbalenya et al. (1989) compared 25 established and putative helicases from *E. coli*, yeast, insects, mammals, viruses, and mitochondrial plasmids, and discussed their involvement in replication, recombination, repair, and transcription (gene expression). In plants, helicases have been studied in chloroplasts (e.g., Cannon and Heinhorst 1990).

### b) DNA Polymerases

At the *Conference on Eukaryotic DNA Replication in Cold Spring Harbor* (1989) it was decided to revise the nomenclature for eukaryotic (which normally means "mammalian") DNA polymerases, and to adopt the Greek letters also for the yeast DNA polymerases, due to the close correlation between many of the mammalian and yeast enzymes. Table 1 shows the present Greek letter nomenclature in relation to the Roman numerals used so

**Table 1.** The revised nomenclature of eukaryotic DNA polymerases (in comparison with the former yeast numerals) and their suggested functions. (Compiled from Burgers et al. 1990; and Thömmes and Hübscher 1990a)

| Polymerase Type | Yeast | Main Suggested Function |
|---|---|---|
| pol α[a] | I | Replication of the lagging strand<br>Completion of Okazaki fragments?<br>Repair of nuclear DNA |
| pol β | – | Repair of nuclear DNA<br>Completion of Okazaki fragments?<br>Recombination? |
| pol γ | m | Replication of mitochondrial DNA |
| pol δ[a] | III | Replication of the leading strand |
| pol ε | II | Repair of nuclear DNA?<br>Replication of nuclear DNA? |
| – | – | Replication of chloroplast DNA |
| – | REV3 | Mutagenic DNA repair |

[a] Both corresponding to pol III of *E. coli*, and gene 43 protein of T4, and UL30 of HSV1.

It can be seen that the list is still incomplete, and that many functions are not completely clear.

far in yeast, and their biological functions (adopted from Burgers et al. 1990; Thömmes and Hübscher 1990a). The attempts to correlate plant DNA polymerases with animal ones are still not very successful (cf. Laquel et al. 1990).

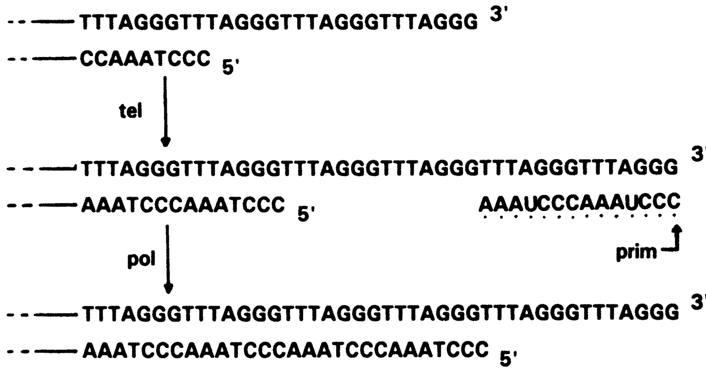
The function of DNA polymerase α (pol α) in replication of the lagging strand, and of polymerase δ (pol δ) in that of the leading strand (see Nagl 1989), as well as the conserved evolutionary properties and their associated factors, have been reviewed by Burgers (1989), Tsurimoto and Stillman (1989), Fairman (1990), and Tsurimoto et al. (1990). Also the 3'->5'exonuclease active site in prokaryotic and eukaryotic DNA polymerases is highly conserved (Bernad et al. 1989). Joyce (1989) studied how DNA travels between the polymerase and exonuclease sites. The characteristics and role of the latest member of mammalian DNA polymerases, pol epsilon, was reviewed by Syväoja (1990) and Bambara and Jessee (1991).

Pausing of DNA polymerases, i.e., the decreased rate or blockage of chain elongation, occurs at sequence specific sites in prokaryotes and eukaryotes. However, termination can, on principle, occur without regard to DNA sequence whenever two replication forks meet. Therefore, the pausing phenomenon needs further investigation; some aspects have been recently studied by Bedinger et al. (1989) and Weisman-Shomer et al. (1989).

The accuracy of DNA primase was studied by Zhang and Grosse (1990). Goodman et al. (1990) and Roberts et al. (1990) summarized the fidelity of DNA polymerase, and Kunkel (1990) reviewed DNA synthesis errors by misalignment.

c) Telomerase

Telomeres are the physical ends of linear chromosomes. Replication of linear DNA molecules by conventional mechanisms leaves gaps at the 5'ends of newly replicated strands, as after removal of RNA primers no 3'-hydroxyls are there. A variety of solutions for replication of DNA termini have, therefore, been proposed during the last decade. Only very recently, after telomeric sequences have been sequenced in a number of organisms, has the probably real mechanism been found. The repeat unit of telomere sequences is highly conserved [reading the strand running 5' → 3' from the end towards



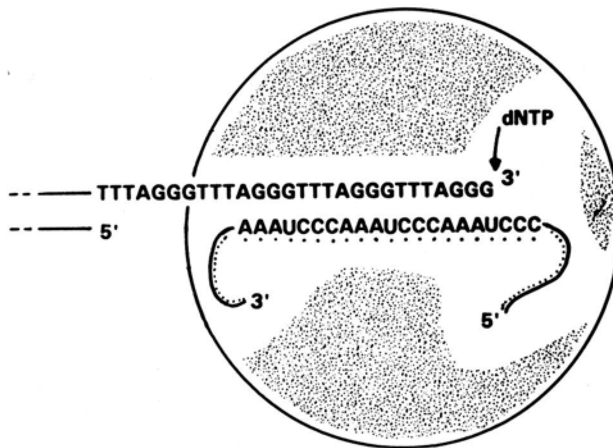




**Fig. 3a,b.** The recent models of replication of telomeric DNA and of the enzyme telomerase. **a** Incomplete end of chromosomal DNA after excision of the last primer from the newly synthesized strand (the sequence given was found in *Arabidopsis* by Richards and Ausubel 1988). Telomerase (*tel*) extends 3'end by nontemplated nucleotide addition. After synthesis of a new primer by primase (*prim*), DNA polymerase (*pol*) extends the incomplete strand by templated DNA synthesis. The resulting chromosome end is complete, but owns an additional single-stranded tail (which may be degraded by nuclease). **b** A schematic model of telomerase extending the single-stranded chromosome end, also showing the RNA component of the enzyme. (After Blackburn 1990). RNA strands are indicated by *dots*

the interior, e.g., 5'-($C_3TA_2$)n–3' in vertebrates including humans (Moyzis et al. 1988; Meyne et al. 1989), and 5'-($C_3TA_3$)m–3' in *Arabidopsis* (Richards and Ausubel 1988)]. A human telomere probe excellently labels the telomeres of *Phaseolus* giant chromosomes (Nagl 1991). Telomerase, a telomere-specific terminal transferase first identified in ciliated protists, provides an excellent candidate for a telomere-specific polymerase (Greider and Blackburn 1985; Zahler and Prescott 1988). This activity can extend the 3'end of the G-rich strand of a variety of telomeric sequences in the absence of a DNA template. The extended 3'OH strand can then serve as a template for primase and conventional polymerase-mediated replication of the complementary strand. Although RNA primer removal would still leave a gap at the end of the 5'strand, no sequence information would be lost, due to the extension of the telomeres. Telomerase was identified as a ribonucleoinprotein that synthesizes multiple telomeric DNA repeats (Fig. 3; Morin 1989; Shippen-Lentz and Blackburn 1989; for reviews see Zakian 1989; Blackburn 1990).

## 6. DNA Amplification

Direct and indirect evidence of DNA amplification in plants has been reviewed in the last issues of this series (Nagl 1985, 1989), and recently by Bassi (1990) and Nagl (1990a); for animals see Hames and Glover (1990). Data have accumulated during the past few years which support the conclusion that changes in the copy number of genes and particularly of repetitive sequences are a widespread phenomenon in the different phases of plant development and that the phenomenon occurs most frequently in plants under stress and in cell cultures.

A very interesting situation was found by Deumling and Clermont (1989) in *Scilla siberica* in response to environmental conditions. During callus culture, several different forms of chromatin loss were observed, such as chromosome elimination, or dramatic reduction in heterochromatin and satellite DNA and other chromatin moieties, resulting in the formation of small chromosomes, etc. However, when regenerated plants were grown in the garden for at least 1 year, the satellite DNA as well as certain coding sequences increased by amplification to 30–40% of their normal proportion, whereas the total DNA had increased by only approximately 15%.

Escandon et al. (1989) followed the fate of five selected genes coding for known, functional proteins (histone H3 and H4, rubisco, 25S rDNA, ubiquitin) during different phases of callus cultures of *Oxalis glaucifolia* and *O. rhombeo-ovata*, differing sevenfold in their 2C nuclear DNA content, i.e., in the percentage of repetitive DNA. While all callus tissues showed significant amplification of the genes relative to differentiated leaf tissue, the pattern of amplification was different for the two species. Furthermore, each gene was amplified to a different degree, suggesting an independent control mechanism. Cuzzoni et al. (1990) analyzed the amplification of a repeated chromosomal DNA sequence in suspension cell cultures of rice, *Oryza sativa*, and found that the amplified DNA is extrachromosomal, probably circular, and comprises about 1% of total rice DNA. The following new report is also available on gene amplification in herbicide-resistant plant cells. Goldsbrough et al. (1990) selected a series of tobacco (*Nicotiana tabacum*) cell lines for growth in the presence of normally lethal concentration of

glyphosate, up to 20 mM. the tolerance was correlated with increased activity and level of the inhibited enzyme, 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS), an increased expression of EPSPS mRNA, and the amplification of the genes encoding this enzyme. Evidently, the genetic modification was stable.

No amplification of the storage protein and several different genes was detected by Johnson and Sussex (1990) in the cotyledon parenchyma of common bean, *Phaseolus vulgaris*, in spite of the high expression of (e.g.) the phaseolin gene and the accumulation of storage proteins. In wheat (*Triticum aestivum*) endosperm, a large segment of DNA (identified by a restriction fragment probe) is apparently even deleted during differentiation of the grain (Tomaszewski et al. 1991). The authors suggested that this deletion is not involved in senescence; similarly, Abeles and Dunn (1990) did not detect loss of DNA fragments by restriction fragment length polymorphism (RFLP) analysis in senescing cotyledon tissue of *Cucumis sativus*.

It should be briefly recalled that amplification is the most frequent evolutionary event that leads to variation of species-specific 2C nuclear DNA amounts in plants (Nagl 1990a, b). There is now increasing evidence that the induction of amplification is closely linked to environmental stress and visits nearly exclusively repetitive DNA (Bassi 1990). New examples have been published for DNA increase with altitude (Porter and Rayburn 1990) and with slow growth habitus (Nagl 1990b). DNA amplification is now also accepted as the main mechanism leading to genome (e.g., satellite DNA) and chromosome (e.g., C band) differences in animals (e.g., Hamilton et al. 1990).

In *Drosophila*, multiple origins have been found to be used in chorion gene amplification (Heck and Spradling 1990). In this well-studied model system, also amplification control elements (ACEs) and amplification enhancing regions (AERs) could be detected and analyzed (e.g., Delidakis and Kafatos 1989; Orr-Weaver et al. 1989). Similar amplification-promoting sequences have also been reported to occur in the ciliates *Stylonychia lemnae* (Wegner et al. 1989) and *Tetrahymena thermophila* (Yu and Blackburn 1990). The effects of such elements may be subsumed under the phenomenon of position effects. Differential amplification of ribosomal RNA genes has been observed during the development of the mosquito, (Park and Fallon 1990) and the house cricket (Tröster et al. 1990). Tissue-specific changes in repetitive DNA in rats might also be caused by amplification, but different interpretations are also possible (e.g., Yokota et al. 1989).

Numerous reports deal with the amplification of protooncogenes and oncogenes in human and animal cancers (e.g., Heilbronn and zur Hausen 1989; Tlsty et al. 1989; Børresen et al. 1990; Somers et al. 1990; Lonn et al. 1991). In these systems much progress has been achieved in the correlation of biochemical data (e.g., from Southern blots) with cytogenetic data, as the amplified DNA is microscopically visible in the form of either an elongated marker chromosome owing a homogenously staining region (HSR), or the presence of small chromatin clumps, the so-called double minutes (DMs) (see, for instance, Lafage et al. 1990; Saint-Ruf et al. 1990; Samaniego et al. 1990; Von Hoff et al. 1990; for short reviews see Schoenlein and Gottesman 1990; Schwab and Amler 1990). These DMs contain circular DNA and are capable of extrachromosomal amplification (Dolf et al. 1991). In plants, amplification is often recognizable in interphase cells by the disproportional increase of heterochromatin, but careful studies of mitotic chromosomes may reveal the existence of phenomena similarly to HSRs and DMs

in plants also. Shang and Wang (1991) found DMs in polytenic callus cells and roots of regenerated wheat in connection with DNA amplification.

Another intensively studied aspect of relevance is tolerance or resistance of organisms against drugs, chemicals, and toxins, as one of the effective molecular mechanism is gene amplification (for a general review see Hayes and Wolf 1990). This phenomenon is of considerable economic importance and often has grave consequences on health. The repeated use of insecticides, herbicides, and chemotherapeutic agents often leads ultimately to their becoming ineffective (for insects see Laval et al. 1989; Georghiou 1990; Mouches et al. 1990, Devonshire and Field 1991; Raymond et al. 1991). In addition, the increasing concentration of pesticides in the environment may cause severe harm to humans (e.g., by water and food uptake) and to the ecosystem.

Most information is so far available from the dihydrofolate reductase (DHFR) gene amplification in methotrexate (MTX)-resistant cells. The locus is well characterized (Hamlin and Ma 1990), as well as the DNA initiation sites (Ma et al. 1990; Vaughn et al. 1990b). According to recent results, amplification takes place throughout the course of the S phase (Caddle and Heintz 1990), and also the replication of amplified genes located on DMs occurs throughout the S phase of the cell cycle (Tlsty and Adams 1990). As already mentioned above for normal initiation of DNA replication, also the origin region of the amplified chromosomal replicon was shown to contain a repetitive element and multiple sequence elements that facilitate DNA bending, DNA unwinding, and the formation of intramolecular triple-stranded DNA (Caddle et al. 1990).

Considering the pollution of air, water, and soil, the resistance to metal ions due to amplification is also of general interest (for examples see Detke et al. 1989; Jensen et al. 1989; Mehra et al. 1990). Another important aspect is very high drug (e.g., hydroxyurea) resistance due to multiple gene amplifications (Hurta and Wright 1990), and the phenomenon of multidrug resistance (MDR), which is associated (at least in part, amplification dependent) with the overexpression of a heterogeneous group of antigenically related membrane phosphoglycoproteins, termed P glycoproteins (for reviews see e.g. Croop et al. 1988; Endicott and Ling 1989).

The dynamics of the eukaryotic genome was also confirmed by studies of DNA amplification. The event, and the presence of amplified regions, evidently decreases the stability of chromosomes (Miele et al. 1989; Ruiz and Wahl 1990). Deamplification (loss) and rearrangements are the consequence (Qumsiyeh and Suttle 1989). On the other side of that genome-chromosome circuit, chromosome breakage is a factor of amplification, deletion etc. (Windle et al. 1991). Hence, these events may deal as good models for genome fluidity in differentiation and evolution processes.

The "amplification" of the complete genome, i.e., polyploidization, in relation to differentiation and evolution was briefly reviewed by Nagl (1990c).

### a) PCR

The term "amplification" is now also generally used for the in vitro propagation of specific DNA sequences by the polymerase chain reaction (PCR) (Mullis 1985; Saiki et al. 1985; Mullis and Faloona 1987). This unfortunately leads to some confusion at a time when key words are selected by computers in all of the common bibliographical data

systems, but computers cannot discern between a biological event and a high-tech method. In any case, this new technology has an enormously high impact on molecular biology, as it is capable of increasing the amount of a target DNA sequence in a sample by synthesizing many copies of the DNA segment. PCR is carried out in discrete cycles, and each cycle of amplification can double the amount of target DNA, so that it is amplified exponentially. One of the prerequisites of that technique was the isolation of Taq polymerase, i.e., the thermostable, exonuclease-deficient, DNA polymerase of high fidelity, isolated from *Thermus aquaticus*. The method and its numerous applications is, however, outside the scope of this chapter (for recent reviews see Arnheim 1990; Linz and Degenhardt 1990).

## 7. Epilogue

With the application of sophisticated molecular biological techniques to eukaryotic systems it becomes clear that "simple" chemical in vitro systems and prokaryotic systems cannot adequately and sufficiently describe the events of DNA replication in higher plants and animals, and still less its regulation. The main differences can be seen in (1) the high number of (rather independent) replicons, (2) their different and varying nuclear environment, (3) the coupling (and sometimes uncoupling) of DNA replication to a series of control points in the cell cycle, and (4) some other additional features like nucleosomes and telomeres. Obviously all of these aspects can be related to the hierarchical structural organization of chromosomes and chromatin in the interphase nucleus.

Another point has won increasing evidence, i.e., the paradigma of the plasticity, fluidy, or dynamic state of the genome, particularly that of higher plants. Almost all of this plasticity originates in noncoding, repetitive DNA, and therefore, should have a functional face. Actually, it is very likely that the variation in repetitive DNA can be the key to the understanding of nearly all the aspects of DNA replication reviewed in this report. The repetitive DNA represents the dominant factor of structural organization of the nucleus, and it exerts (by mass) nucleotypic effects of growth and (through structure) position effects onto genes and replicons. By this, variation in repetitive DNA (and of a few genes) in adaptation to the environment determines the process of evolution, and the variation within an organism is one of the regulatory circuits of differentiation and morphogenesis.

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