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Biochemistry

Increased intracellular concentration of an initiator protein markedly reduces the minimal sequence required for initiation of DNA synthesis

(filamentous phage cloning vectors/origin mutants/gene *II* and gene *V* proteins/translational control/DNA–protein interactions)

GIAN PAOLO DOTTO* AND NORTON D. ZINDER†

The Rockefeller University, New York, NY 10021

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ABSTRACT **One of the most common sites used for cloning in the filamentous phages f1, fd, and M13 lies within the phage "functional origin," a sequence of 140 nucleotides that is required for phage replication. Even small insertions (four nucleotides) at this location severely reduce origin function. Secondary *trans*-acting mutations in the phage genome are necessary to restore efficient replication. One of these mutations, present in one of our cloning vectors, R218, has been fully characterized. It consists of a regulatory mutation within gene *V* that leads to a marked increase in the intracellular level of the phage gene *II* protein, the "initiator" of viral replication. Increased gene *II* protein production is sufficient to reduce the minimal sequence required for a functional origin to only 40 nucleotides, while the remaining 100 (containing the cloning site) become entirely dispensable. The general implications of these findings are discussed.**

All DNA molecules contain in their nucleotide sequence regulatory regions that interact with specific proteins to fulfill a variety of functions such as DNA replication and transcription. The specificity of these DNA–protein interactions is essential for regulation of the various functions and can be drastically affected by small mutations in either the nucleotide sequence or the protein(s) involved. Two well-studied cases are (*i*) simian virus 40 origin of DNA replication and tumor (T) antigen (1) and (*ii*) λ phage DNA with its repressor (2). In this communication, we present another way in which DNA–protein interactions can be altered: with bacteriophage f1, a simple increase in intracellular concentration of the "initiator" protein that recognizes the phage origin of DNA replication is sufficient to reduce to less than one-third the minimal sequence required as a replication origin. The general implications of these findings will be discussed.

DNA replication of the filamentous, single-stranded DNA phage f1 (also phages fd and M13) has been studied in great detail. After entering the bacterial cell, the f1 viral (+) strand is converted by host enzymes into a double-stranded, circular, superhelical molecule (replicative form I, RFI) (3). Viral (+)-strand synthesis is then initiated by the viral gene *II* protein, which introduces a nick at a specific site [(+) origin] on the (+) strand of the RFI molecule (4). Elongation of the 3′ end of the nick is accompanied by displacement of the old viral strand by a rolling-circle mechanism (5). In addition to its role in initiation, gene *II* protein is also able, after one round of (+)-strand synthesis, to cleave the nascent single-stranded tail from the replicative intermediate and seal it to form a covalently closed circle (6). The final products are a closed, single-stranded molecule and a closed, double-stranded RF molecule (7). In the early stages of phage infection, the single-stranded circular DNA formed serves as template for the synthesis of more RF molecules. In later stages, the single strands interact with the viral gene *V* protein (8, 9) and subsequently are packaged into viral particles. Gene *V* protein, in addition to its role as a single-stranded DNA binding protein, is able to regulate the amount of gene *II* protein present inside the cell by depressing the translation of gene *II* mRNA (10, 11). In this way, gene *II* protein is normally maintained inside the infected cells at low levels.

The f1 genome consists of 10 genes and an intergenic region (IG) 508 nucleotides long that does not code for any known protein (12) but contains the phage packaging (morphogenetic) signal (13, 14) and origin of DNA replication (15). The phage "functional origin" of DNA replication has been defined as the minimal f1 sequence that, when harbored in a plasmid (ori-plasmid), allows it to enter the f1 mode of replication when helper phage is present (13). Its biological activity can be measured, upon f1 infection, in three ways: (*i*) stimulation of plasmid RF synthesis; (*ii*) interference with f1 DNA replication; and (*iii*) formation of virion-like particles that contain exclusively plasmid single-stranded DNA and transduce resistance to antibiotics (transducing particles). Such a region is located in the middle of the IG and extends for 12 nucleotides on the 5′ side of the f1 (+) origin (16) and for more than 100 on its 3′ side (13, 17) (see Fig. 1). It can be divided into two domains (18) (Fig. 1): A (40 nucleotides long) and B (100 nucleotides long). Disruption of domain B by deletions or insertions causes a drop in biological activity of the origin to 1/100th (as measured in the ori-plasmid system), whereas disruption of domain A causes a drop to 1/10,000th or more. Therefore, domain A can be considered the "core" of the functional origin. Domain A contains three partially overlapping sequences essential for viral (+)-strand replication (18) (Fig. 1): (*i*) the gene *II* protein *in vitro* recognition sequence; (*ii*) a sequence required for initiation of (+)-strand synthesis; and (*iii*) a sequence required for its termination. Domain B contains a sequence required exclusively for initiation (18) (Fig. 1).

Given their filamentous structure, the filamentous phages, f1, fd, and M13, can accommodate in their virions DNA molecules much longer than their genomic unit length. This unique property has allowed the development of these phages as single-stranded cloning vectors (19). All of the cloning sites that have been introduced in these phages lie in the IG; in particular, the one most frequently used maps well

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Abbreviations: RF, replicative form; IG, intergenic region.

*Present address: Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

†To whom reprint requests should be addressed.

within the phage "functional origin" (19–21) (Fig. 1). Our efforts to understand how it is possible to insert large stretches of foreign DNA in such a crucial region of the phage genome without disrupting its replicative function led to the findings reported here.

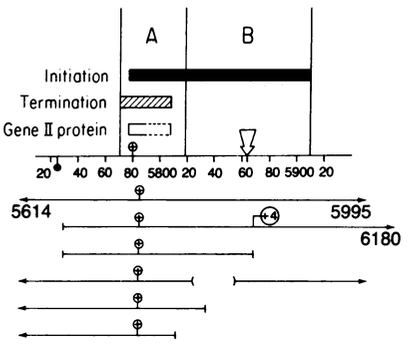
MATERIALS AND METHODS

The bacterial strain used was *Escherichia coli* K-38 (22). f1 phage was from our laboratory stock. The construction of mutant phage R209 has been described (21). R218 is a clear-plaque derivative of R209 isolated by J. D. Boeke in our laboratory. R13, R132, R133, and R163 are from our laboratory stocks (12, 22). Plasmids pD38, pD48, Δ+41,70, and Δ+29 have been described (18). Plasmid pD41 contains the f1 *Hae* III G fragment inserted at the *Eco*RI site of pBR322. Plasmid pD49 contains the R218 *Hae* III G + D fragment (the *Hae* III G/D site is substituted in this phage by a unique *Eco*RI site) inserted at the *Bam*HI site of pD37, a pBR322 derivative previously described (18). Plasmid pD2 has been described (23). DNA fragments were purified by electrophoresis on low-melting agarose gel (FMC, Rockland, ME) in 40 mM Tris·HCl, pH 7.8/5 mM NaOAc/1 mM EDTA. Bands were sliced out and incubated at 65°C for 1–2 hr. Equal volumes of equilibrated phenol were then added, and the samples were kept in ice for 30–60 min prior to extraction of the aqueous phase. A second phenol extraction was performed, and the DNA was recovered by precipitation with ethanol. *In vitro* recombination experiments were performed by combining purified fragments in equimolar amounts (at 50 μg/ml) in a ligation reaction with T4 ligase (New England BioLabs) at 4°C for 12 hr. Conditions for all other DNA manipulations (13, 18) and marker rescue experiments (24) have been described. Nucleotide sequence determinations were performed as described by Sanger *et al.* (25). The conditions for protein labeling, immunoprecipitation, and $NaDodSO_4$/PAGE also have been described (26–28).

RESULTS

R218 is a single-stranded cloning vector derived from a clearer plaque of R209, which was obtained by inserting a unique *Eco*RI site (4 nucleotides) at the phage f1 *Hae* III G/D site (21). This site (map position 5867) lies within domain B of the phage "functional origin" (map positions 5769–5909) (18) and is the same site used in the construction of the M13-derived Mp1 vector (20). R218 and Mp1, with or without inserted sequences, replicate very efficiently, their yields being comparable to those obtained with f1 (or M13) wild type.

In an attempt to understand how it is possible to insert foreign DNA in domain B without disrupting replicative function, we used the same chimeric ori-plasmid system mentioned above. The R218 functional origin (*Hae* III G + D fragment) (Fig. 1) was cloned at the *Bam*HI site of pD37, a pBR322 derivative previously described (18). When the resulting plasmid (pD49) was tested with phage f1 wild type as helper, the R218 origin (by measuring the ability of pD49 to interfere with f1 replication and yield transducing particles) was at most 1/100th as active as a "wild-type" origin. However, the R218 origin contained in pD49 was fully active when R218 instead of f1 wild type was used as helper phage (Fig. 1). More surprisingly, the same result was observed with other ori-plasmids in which only domain A (the "core" of the functional origin) is retained intact, while domain B is partially or totally deleted (Fig. 1). These plasmids retained only 1% of biological activity when tested with f1 wild type but were fully active when tested with R218. Ori-plasmids in which domain A was disrupted showed a very drastic loss of biological activity with both f1 and R218 as helper (Fig. 1, Δ+29). Thus, it appears that, with R218 as helper, the core of the functional origin (domain A) remains essential, while



	BIOL. ACTIVITY f1	R218
pD38	100%	100%
pD49	1%	100%
pD41	1%	100%
Δ+41,70	1%	100%
pD48	1%	100%
Δ+29	<0.01%	<0.01%

FIG. 1. (*Left*) Map of the phage f1 functional origin and of the f1 fragments present in the various plasmids. The location of the gene *II* protein recognition sequence and of the signals for initiation and termination of viral strand synthesis are indicated (16, 18). Also shown are the domains into which the f1 functional origin can be divided (18). ⊕, Gene *II* protein nicking site [(+) origin]; ♦, *Hae* III sites; ▽, site used in the construction of R218(R209) and Mp1 (19). Numbers indicate nucleotide positions on the f1 map (29, 30). Aligned with it are the f1 fragments present in the various chimeric plasmids used in this study; arrows indicate that the fragments extend up to the indicated positions, the blank space between brackets in Δ+41,70 indicates the deleted sequence (18), and ⊕ shows the location of the four-nucleotide insertion in the R218 origin cloned in pD49. (*Right*) The biological activity of the various origins measured with either f1 or R218 as helper phage is defined in each case as the ratio between the yield of transducing particles obtained with a certain chimeric plasmid and that obtained with pD38, the biological activity of which is arbitrarily set at 100%. The yield of transducing particles was normalized in each case to the yield of phage, given the interfering activity of the various plasmids. For instance, from cells harboring pD48 and infected with f1, the recovered phage stock contained 1×10^{12} plaque-forming units (pfu)/ml and 1×10^{10} transducing particles per ml (transducing particles-to-pfu ratio, 0.01). When R218 instead of f1 was used, the phage stock obtained from the same cells contained 2×10^{10} pfu/ml and 3×10^{10} transducing particles per ml (transducing particles-to-pfu ratio, 1.5).

the rest (domain B) is totally dispensable, at least as measured in this chimeric plasmid system. Similar results were obtained with Mp1 (unpublished results).

To verify the possibility that for R218 domain B is dispensable even in the phage, the following experiment was performed. R218 DNA was cleaved with *Eco*RI (the *Eco*RI site in R218 maps within domain B), extensively digested with exonuclease BAL-31, and recircularized prior to transfection of *E. coli* K-38 cells. Normal-size plaques were recovered; in the several cases examined, the phage DNA contained deletions up to 200 nucleotides that destroyed domain B but did not affect phage viability.

The striking difference between phage f1 and R218 (or Mp1) in the minimal sequence required as a functional origin might be due to two possibilities. (*i*) The R218 origin, or any other origin in which domain B is disrupted, might be "weaker" than an intact origin but might be adequate to ensure, by itself, reasonable yields of phage. However, when there are two phage origins in competition with each other (one of the chimeric plasmid and the other of the helper phage), the presence of an intact origin might render a "weaker" one inactive. (*ii*) The R218 origin, or any other origin in which domain B is disrupted, would be active both in the plasmid and in the phage only if there were a compensating mutation in some other part of the phage genome.

To distinguish between these two possibilities, we performed *in vitro* recombination experiments as summarized in Fig. 2. Both f1 and R218 RFI DNAs were cut with several restriction enzymes in such a way that in each case the phage genome was divided into two fragments. The fragments were purified by agarose gel electrophoresis and added back to-

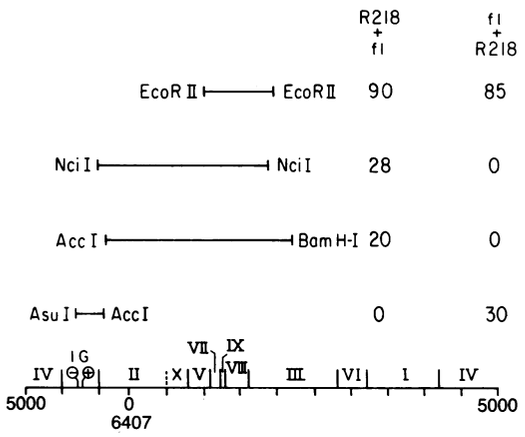


FIG. 2. *In vitro* recombination experiments. The lowest line is the map of the f1 genome (12). Roman numerals are f1 genes; X is the region of gene *II* coding for protein X; IG is the intergenic region; and ⊕ and ⊖ show the origin of synthesis of viral (+) and complementary (−) strands. Nucleotide positions (29, 30) are also indicated (numbers and short unlabeled bars). Superior to the map are lines showing the smaller (A) of the two f1 fragments generated in each case by restriction enzyme digestion of f1 or R218 DNA. Next to them are the numbers of plaques recovered after transfection of K-38 cells by using the combination of either the R218 smaller (A) fragment with the f1 larger (B) fragment or vice versa. In all cases, numbers are an average of at least two experiments. Not shown (but comparable to the positive numbers) are the numbers of plaques obtained with the homologous combinations of fragments: R218 fragments A + B and f1 fragments A + B.

gether in all possible combinations: R218 + R218; f1 + f1; R218 + f1; f1 + R218. After ligation, the various combinations were used for transfection of *E. coli* K-38 cells, and plaque production was taken as a measure of their viability.

In a first experiment, phage DNA was doubly digested with *Asu* I and *Acc* I so that two fragments were generated (Fig. 2): a smaller one (fragment A), containing the phage functional origin and the amino-terminal part of gene *II*, and a larger one (fragment B), containing the rest of the phage genome. All but one of the possible combinations of these fragments yielded a good number of plaques (Fig. 2). The R218 fragment A (containing the R218 origin) together with the f1 fragment B (containing the rest of the genome) yielded no plaques. Thus, it appears that in phage the R218 origin is inactive unless a second mutation in some other part of the phage genome (R218 fragment B) can rescue it. This finding was confirmed by other recombination experiments.

When smaller fragments (A) obtained from f1 DNA by digestion with either *Nci* I or *Acc* I/*Bam*HI (which contain most of gene *II*, genes *V*, *VII*, *IX*, *VIII*, and part of gene *III*) (Fig. 2) were combined with their complementary larger fragments (B) derived from R218 (which contain the origin, part of gene *III*, and genes *VI*, *I*, and *IV*), no viable phage could be recovered (Fig. 2). On the other hand, when the smaller f1 fragment generated by digestion with *Eco*RII (which contains part of gene *V*, genes *VII*, *IX*, and *VIII*, and part of gene *III*) (Fig. 2) was combined with the larger fragment derived from R218 (which contains the rest of the phage genome), the yield of plaques after transfection was good (Fig. 2).

These studies demonstrate that there is a second mutation in the R218 genome that is able to rescue the R218 origin. Comparison of the various combinations of fragments and their viability indicates that such a mutation maps somewhere in either gene *II* (excluding its amino-terminal part) or the amino-terminal part of gene *V* (Fig. 2).

We previously had cloned the R218 gene *II* in a plasmid (pD2) so that large amounts of active gene *II* protein were produced (23). We cloned gene *II* from R218, not from f1 wild type, because of its unique *Eco*RI site that lies just upstream from the gene *II* promoter. K-38 cells harboring plasmid pD2 [K-38(pD2) cells] are able to efficiently rescue gene *II* amber phages by complementation (23). On the assumption that the mutation in R218, which is able to rescue the R218 origin, maps in gene *II*, it was reasonable to assume that K-38(pD2) cells would be able to rescue the nonviable recombinants of the experiments described above. In the combination of the *Acc* I–*Bam*HI f1 smaller fragment with the corresponding R218 larger fragment, most of the genome (including the origin) comes from R218, but genes *II* and *V* are derived from f1 (Fig. 2). Upon transfection of K-38 cells, no plaques could be detected (Fig. 2). However, when the same combination of fragments was used to transfect K-38(pD2) cells, plaques could be obtained, similar in number to those obtained with the complementary viable combinations. Thus, the gene *II* clone with its large amount of gene *II* protein could rescue the R218 origin; this suggested, but did not prove, that the mutation lies in gene *II*.

The recombinant phage (R218/f1) that could be recovered from the K-38(pD2) cells was plaque-purified and found to grow as efficiently as f1 wild type or R218 on K-38(pD2) cells but at most 1/10th as efficiently on K-38 cells. When plated on K-38, R218/f1 gives turbid plaques that are difficult to see and are reminiscent of the original phage R209. This property was exploited to localize the R218 mutation by marker rescue. R218/f1 single-stranded DNA was hybridized to purified R218 restriction fragments, and the various combinations were tested for their ability to produce normal clear plaques on K-38 cells.

The R218 fragments that were "active" in this assay are shown in Fig. 3. The results were all consistent with one another but, contrary to expectations, indicated that the R218 mutation lies in gene *V*, not in gene *II*. For example, the R218 *Hha* I fragment M, which is entirely within gene *V*, is fully proficient in rescuing the R218/f1 phage (Fig. 3). The sequence of this region was determined, and a single nucleotide change was found (a C → T transition at position 901)

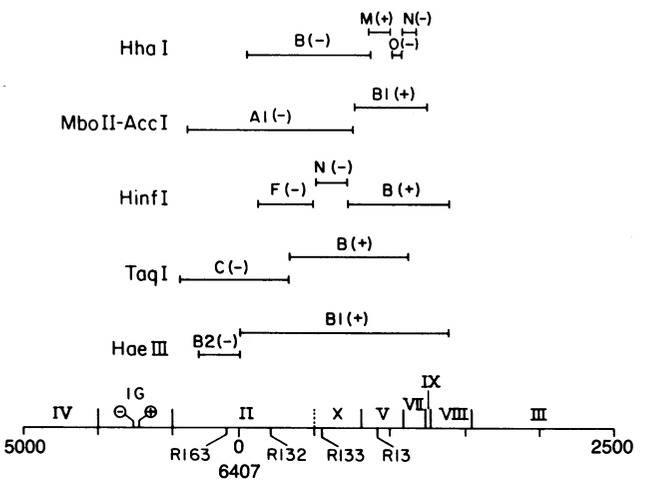


FIG. 3. Marker rescue experiments. The lowest line is the map of the f1 genome in the region of interest. Symbols are as in Fig. 2. The positions of amber mutations in gene *II* and gene *V* are also indicated. Phage containing these mutations (R163, R132, R133, and R13) were used in marker rescue experiments as controls to assess the purity of the various fragments. Superior lines to the map are restriction fragments derived from R218 DNA and used to rescue the R218/f1 phage; (+) and (−) refer to the ability of the fragments to rescue R218/f1. Positive fragments induced a 100-fold or more increase in clear plaque production over background. R218 has, relative to f1, an additional *Mbo* II site at position 1225. No obvious phenotype is associated with it.

that would result in an Arg → Cys substitution at position 21 in the gene *V* protein. The sequence of an overlapping region of the R218 genome had been determined independently by Wilder Fulford in our laboratory and the C → T change at position 901 was the only change found.

Gene *V* protein, in addition to its activity as a single-stranded DNA binding protein, is also responsible for regulating the level of gene *II* protein inside the cell. To reconcile the fact that f1 is rescued by the R218 mutation as well as by the gene *II* clone and that the R218 mutation lies in gene *V*, not in gene *II*, one might assume that the gene *V* mutation (V_{218}) is a regulatory mutation such that large amounts of gene *II* protein are synthesized.

To compare the amounts of gene *II* protein produced with R218 and f1, exponentially growing K-38 cells were infected with various phages and, after 20 min, were pulse-labeled with [^{35}S]methionine for 2 min. Samples were immunoprecipitated with specific antiserum against gene *II* protein and analyzed by NaDodSO$_4$/PAGE. Cells infected with a gene *V* amber phage (lane 5) contained large amounts of gene *II* protein and also of X protein, a protein coded for by the last third of the gene *II* sequence. This finding is consistent with the fact that gene *V* amber mutants under nonpermissive conditions are known to overproduce the gene *II* and X proteins (10, 11). Cells infected with R218 (lane 6) contained gene *II* and X proteins in amounts similar to those observed with the gene *V* amber mutant. These same proteins were present in much lower amounts in cells infected with phage f1 (lane 4). Even if the sample from f1-infected cells was doubled (lane 3), the amounts of gene *II* and X proteins that could be detected remained significantly below those detected with the gene *V* amber mutant or R218. One can estimate that the over-production of gene *II* protein ranges between 5- and 10-fold.

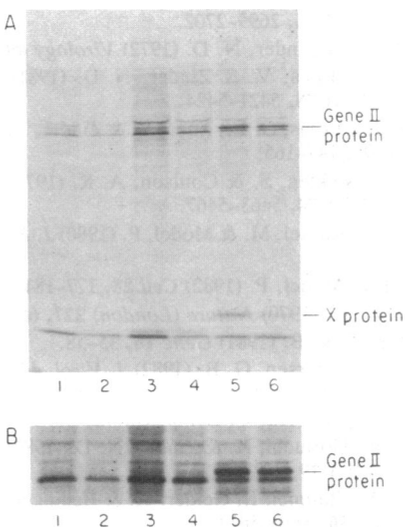


FIG. 4. Gene *II* protein production in cells infected with various phages. Exponentially growing K-38 cells were infected with various phages at a multiplicity of infection of 100. After 20 min, cells (0.2 ml) were labeled for 2 min with 10 μCi (1 Ci = 37 GBq) of [^{35}S]methionine (10^3 Ci/mmol). After precipitation with trichloroacetic acid, samples were immunoprecipitated with gene *II* protein antiserum (provided by R. Webster) essentially as described by Russel and Model (27). Samples were then analyzed by electrophoresis in NaDodSO$_4$/12.5% acrylamide gels (*A*). The same samples were also electrophoresed in a NaDodSO$_4$/7% acrylamide gel to better resolve the region around the gene *II* protein (*B*). The positions of the gene *II* and X proteins are indicated. Lanes: 1, uninfected control; 2, infection with R132 (gene *II* amber mutant); 3 and 4, infection with f1 wild type (lane 3 contains twice the amount of sample used in lane 4); 5, infection with R13 (gene *V* amber mutant); 6, infection with R218.

Samples from the experiment described above were run directly (not immunoprecipitated) on a 22% acrylamide/urea gel (data not shown) to determine the amounts of gene *V* protein produced with the various phages. Although no gene *V* protein was detectable in gene *V* amber-infected cells, good levels of gene *V* protein were present in both f1- and R218-infected cells. Thus, the gene *V* mutation of R218 does not affect the production and/or stability of the gene *V* protein but seems to be a true regulatory mutation, whose only apparent effect is to increase significantly the levels of gene *II* protein inside the cell.

DISCUSSION

The filamentous phages f1, fd, and M13 are often used as single-stranded cloning vectors (19). One of the most common sites for cloning (20, 21) lies within the phage "functional origin of replication," which consists of a sequence of about 140 nucleotides specifically required for the initiation and termination of viral strand synthesis (18, 31, 32). Paradoxically, we found that the insertion of as few as four nucleotides into this site results in a phage whose origin functions weakly. The explanation for the good growth of the cloning vectors probably lies in their containing compensatory mutations elsewhere in their genome.

This is illustrated by one of our cloning vectors (R209) in which an *Eco*RI site (four nucleotides) had been placed in this crucial region. Quickly, a further mutation was selected that restored good growth (R218). The effect of this mutation is quite surprising in that it not only overcomes the negative effect of the four-nucleotide insertion in the phage origin but also renders a large part of the origin itself altogether dispensable. Of the 140 nucleotides that constitute the f1 functional origin, only 40 (domain A) remain essential, while the rest (domain B) are no longer required.

The mutation in R218 was fully characterized. It consists of a nucleotide substitution within gene *V* (V_{218}), leading to a single amino acid change in the gene *V* protein. The mutated protein presumably retains its single-stranded DNA binding activity (R218 gives normal yields of phage) but loses its ability to repress translation of the viral gene *II* mRNA. Because the two functions of the gene *V* protein have been dissociated, they are probably independent of each other. In this respect, *in vitro* studies with purified proteins will be of particular interest.

The V_{218} mutation drastically reduces the minimal sequence required as a replication origin. The increased levels of gene *II* protein inside the cell caused by this mutation could account for such an effect. Support for this hypothesis is given by the fact that a clone that overproduces gene *II* protein (pD2) can complement phages with mutated origins. In addition, preliminary results with other clear-plaque derivatives of R209 and with Mp1 indicate that a variety of other mutations with effects on the origin similar to V_{218} are possible. Some of them map in the gene *II* promoter region and, like V_{218}, lead to overproduction of gene *II* protein. However, others lie in the gene *II* structural region and seem to have no effect on its expression. A better characterization of these mutations is still required, but it already appears that changes in either amount or kind of gene *II* protein inside the cell can have profound effects on the phage DNA synthesis.

Gene *II* protein is an endonuclease that nicks the f1 RFI molecule at a specific site at the onset of viral strand replication (4) and, after one round of synthesis, cleaves the nascent strand (33) and seals it to form a covalently closed, single-stranded circle (6). Using an *in vitro* nicking assay, we have shown previously that the gene *II* protein recognition sequence is contained within the f1 functional origin and extends from not more than four nucleotides on the 5′ side of the gene *II* protein nicking site (16) to 11–29 nucleotides on

its 3′ side (18) (Fig. 1). The *in vitro* recognition sequence of gene *II* protein overlaps with two other sequences that are required *in vivo*, respectively, for the initiation and termination of viral strand synthesis (34). The sequence required for termination (termination signal) contains the gene *II* protein recognition sequence and extends for eight more nucleotides on its 5′ side (18, 32) (Fig. 1). The sequence required for initiation (initiation signal) includes the gene *II* protein recognition sequence and extends for 100 more nucleotides on its 3′ side (18, 32) (Fig. 1). The gene *II* protein recognition sequence and the termination signal are entirely contained within domain A, the "core" of the functional origin, whereas the initiation signal consists of essentially the entire functional origin, including both domains A and B (18) (Fig. 1).

The fact that increased levels of gene *II* protein inside the cell render domain B dispensable raises the question of the nature of the interactions that occur between domain B (and the initiation signal) and gene *II* protein. It is conceivable that a specific sequence, such as domain B, might act as an entry site for the binding of gene *II* protein to the DNA. However, this possibility is rather unlikely because *in vitro*, even with limiting amounts of gene *II* protein, no difference could be detected in the efficiency of nicking of various substrates either containing or lacking domain B (18).

Alternatively, domain B might constitute the site where the replication fork is formed. The role attributed to gene *II* protein for the initiation of (+)-strand synthesis is the introduction of a specific nick at the (+) origin. It is, however, entirely possible that this protein is also actively involved in the formation of the new replication fork. For the "maturation" and initial movement of the fork, the presence of a specific sequence (domain B) might be required. An increased concentration of gene *II* protein might render such a sequence unnecessary by altering, either directly or indirectly, the DNA–protein interactions involved in the initiation process. It will be of great interest to see whether the requirement for these specific interactions, as suggested by these *in vivo* studies, can be reproduced using *in vitro* conditions.

The effects of increasing concentrations of a protein on its ability to interact with specific nucleotide sequences have been carefully studied by using the λ phage repressor–operator system (2). In that system, even in the presence of large amounts of repressor, DNA–protein interactions retain a high degree of specificity (2). In contrast, we have shown here that the stringency of DNA–protein interactions can be drastically altered by a simple increase in the intracellular concentration of one of the protein(s) involved. Our findings may be of general significance and might even bear on recent speculations concerning the role of increased production of certain key cellular substances in disrupting the control of cell growth (35).

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