Volume 212, number 1, 10–14 FEB 04397 February 1987

*Hypothesis*

# A plausible mechanism for large-scale chromosomal DNA amplification in streptomycetes

Michael Young and John Cullum*

*Department of Botany and Microbiology, University College of Wales, Aberystwyth SY23 3DA, Wales* and **Department of Biochemistry and Applied Molecular Biology, UMIST, PO Box 88, Manchester M60 1QD, England*

Received 13 October 1986; revised version received 21 November 1986

Recent advances in our understanding of the structure of highly amplified DNA sequences in *Streptomyces fradiae* and *lividans* have enabled us to formulate a possible mechanism by which amplification may occur. An essential feature of the model is the generation of an amplification precursor, which comprises a circularised copy of the DNA to be amplified, attached to one arm of the chromosome by a replication fork. Multiple copies of the amplifiable DNA are generated by rolling circle replication. The model adequately accounts for many features of gene amplification in these two species, including the tendency for deletions to occur to one side, but not the other, of the amplified DNA.

Gene amplification; Amplification mechanism; (Streptomycetes)

## 1. BACKGROUND

Chromosomal DNA amplification is an extremely widespread phenomenon. In many of the prokaryotes in which it has been documented, e.g. *Salmonella typhimurium* [1], *Escherichia coli* [2,3], *Bacillus subtilis* [4–10] and *Streptococcus pneumoniae* [11], small-scale amplification (establishment of 5–50 copies of an amplified DNA segment per genome) has been observed. Although individuals with amplified DNA arise spontaneously [6,7], they are comparatively rare and, generally speaking, have been detected after the imposition of deliberate selection for increased dosage of a gene product encoded by the amplified DNA segment [2,3,5]. Although little is yet known about the mechanism of amplification, various models have been adduced. Those favoured by many investigators are the occurrence of repeated cycles of either (i) unequal crossing over between sister chromosome arms during replication [12], or (ii) excision and reintegration of the DNA segment undergoing amplification [13,14]. Circularised excision products, whose existence as amplification intermediates is predicted by the latter model, have apparently been observed by some investigators [14,15]. Both models predict that amplification should proceed in a gradual, stepwise fashion and there is experimental evidence to support this [5,14].

The streptomycetes are notorious for their genetic instability, an aspect of which is the frequent amplification of chromosomal DNA sequences (reviewed by Cullum et al. [16]). There are three important differences between the amplification events that occur in streptomycetes and those

Correspondence address: M. Young, Dept of Botany and Microbiology, University College of Wales, Aberystwyth SY23 3DA, Wales



*Published by Elsevier Science Publishers B.V. (Biomedical Division)*
00145793/87/$3.50 

that have been studied in other prokaryotes. Firstly, variants harbouring amplified DNA segments arise frequently and may be maintained in the absence of any obvious selective pressure [17]. Secondly, amplification events are usually accompanied by the occurrence of extensive deletions [17–19]. Thirdly, most of the variants that have been studied show large-scale amplification (100–500 copies of the amplified DNA segment per genome). It seems inherently improbable that variants with such highly amplified DNA segments could appear by the operation of either the unequal crossing over or the excision/reintegration mechanisms mentioned above. As has been suggested [17,19–21], a mechanism that includes a phase of replication would seem more plausible. Many of the characteristic features of DNA amplification in eukaryotes can be accounted for by what Schimke [22] has termed a saltatory replication mechanism, in which repeated rounds of replication are initiated from one replication origin during a single cell cycle. This would generate an amplification intermediate that has been likened to an 'onion skin', in which 'localized polytenization' has effectively occurred [23,24]. There are difficulties in relating this mechanism to amplification in streptomycetes. For example, unless the activation of otherwise silent origins is invoked, amplification would be restricted to sequences close to the normal origin of replication of the chromosome. Another discrepancy is that resolution of the onion skin intermediate leads to the establishment of a heterogeneous collection of DNA sequences in the amplified region in eukaryotes [23,24], whereas reiterated sequences in streptomycetes (and other prokaryotes) form regular, tandemly repeated arrays.

One possible way in which to generate such regularly repeated structures would be by rolling circle replication [25] of a suitable amplification precursor. Recent work on the detailed structure and organisation of DNA sequences that are prone to amplification [19,21,26–28] has led us to propose a simple mechanism whereby amplification could occur. It not only accounts for the sudden establishment of multiple tandemly repeated copies of the amplified DNA segment, but also serves to explain the simultaneous occurrence of certain types of deletions that often accompany amplification events.

## 2. ORGANISATION OF AMPLIFIED DNA IN *STREPTOMYCES FRADIAE* AND *S. LIVIDANS*

Hershberger and his colleagues [21,27,28] have cloned and analysed a 10.5 kbp DNA segment that is amplified in certain strains of *S. fradiae*. In the parental (non-amplified) strain the amplifiable sequence comprises a unique internal sequence of 8.3 kbp flanked by two directly repeated 2.2 kbp elements. In strains harbouring amplifications, the DNA sequence that is reiterated comprises the internal sequence, together with one copy of the flanking element. The flanking element is also present at two other sites in the parental strain [21].

An amplifiable DNA segment from *S. lividans* has also been cloned and analysed by Altenbuchner and Cullum [19,26]. Its organisation is strikingly similar to that of the amplifiable DNA segment in *S. fradiae*. The internal sequence in this case comprises 4.7 kbp and the flanking element about 1.0 kbp, and in the parental strain there are two copies of the 4.7 kbp sequence flanked by, and interspersed with, three copies of the 1.0 kbp element. From the parental strain a DNA segment was cloned which contained the sequences that undergo amplification, together with adjacent non-amplifiable DNA on either side. These adjacent sequences were used as hybridization probes to demonstrate that strains harbouring amplifications suffered concomitant deletions of DNA on one side of the amplified region but not the other.

## 3. A POSSIBLE AMPLIFICATION MECHANISM

In formulating the model (fig.1), we wished to account for the following essential features of amplification in streptomycetes:

(i) An internal DNA segment bordered by directly repeated elements can undergo amplification.
(ii) Amplification may occur in a single step to yield a large number of copies of the amplified DNA.
(iii) The amplified DNA comprises the internal DNA segment plus one copy of the repeated element and is established as a tandemly repeated array.
(iv) DNA sequences on one side, but not the

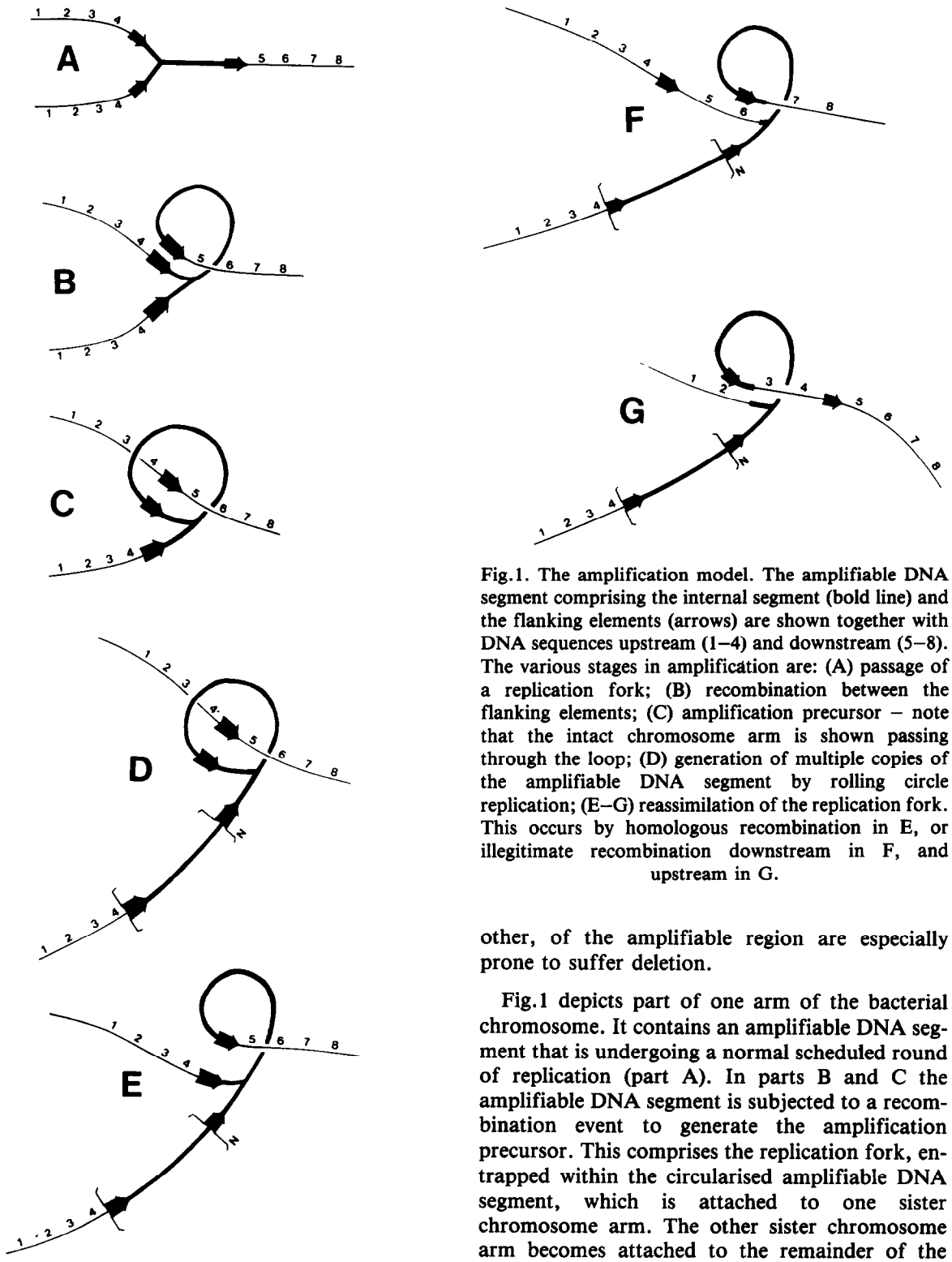


Fig.1. The amplification model. The amplifiable DNA segment comprising the internal segment (bold line) and the flanking elements (arrows) are shown together with DNA sequences upstream (1–4) and downstream (5–8). The various stages in amplification are: (A) passage of a replication fork; (B) recombination between the flanking elements; (C) amplification precursor – note that the intact chromosome arm is shown passing through the loop; (D) generation of multiple copies of the amplifiable DNA segment by rolling circle replication; (E–G) reassimilation of the replication fork. This occurs by homologous recombination in E, or illegitimate recombination downstream in F, and upstream in G.

other, of the amplifiable region are especially prone to suffer deletion.

Fig.1 depicts part of one arm of the bacterial chromosome. It contains an amplifiable DNA segment that is undergoing a normal scheduled round of replication (part A). In parts B and C the amplifiable DNA segment is subjected to a recombination event to generate the amplification precursor. This comprises the replication fork, entrapped within the circularised amplifiable DNA segment, which is attached to one sister chromosome arm. The other sister chromosome arm becomes attached to the remainder of the

chromosome arm that lay downstream from the replication fork at the time of recombination. Note that the occurrence of a similar recombination event in the absence of a replication fork would simply lead to excision of the amplifiable DNA segment. Further replication (part D) by a rolling circle mechanism [25] generates multiple tandemly repeated copies of the amplifiable DNA segment.

Resolution of this amplification intermediate to restore the replication fork to its original configuration can occur in several ways. In part E it has undergone homologous recombination with the region from which it originated, in which case the amplified DNA becomes established in the chromosome without any alteration in the adjacent DNA. In part F illegitimate recombination has occurred in a region downstream from the site where the initial recombination event occurred. This results in deletion of the sequences that lay downstream in the parental strain. In part G illegitimate recombination has occurred in a region upstream from the site where the initial recombination event occurred. This leads to establishment of the amplified DNA flanked by directly repeated copies of sequences that lay upstream in the parental strain.

There are a variety of other possible ways in which the replication intermediate shown in part D can be resolved. Some of these would lead to loss of the replication fork from the chromosome arm. Assuming that replication of the circular chromosome is normally bidirectional, this would result in a chromosome with only one replication fork. Events of this nature may be lethal, since termination of chromosome replication cannot occur normally. In any case, it is quite probable that events of the kind shown in fig.1 are favoured. This is because after recombination has occurred (parts B and C), the chromosome arm from which the replication fork was withdrawn may pass through the circularised amplification precursor, as shown. Hence, as amplification proceeds the loop will remain in intimate association with the chromosome arm from which it was withdrawn and there will be ample opportunity for it to be reassimilated.

## 4. IMPLICATIONS OF THE MODEL

The model adequately accounts for the four essential features of amplification in streptomycetes that are enumerated above. It is especially interesting that it predicts the occurrence of deletions on one side, but not the other, of the amplified DNA. That such events appear to be commonplace in *S. lividans* may fortuitously result from the selection of $Arg^-$ strains for investigation; it is tempting to speculate that the *argG* gene lies on the side of the amplifiable DNA segment that is prone to deletion in this organism. Events such as those shown in parts E and G may only be detectable among $Arg^+$ strains. Alternatively, there may be a natural tendency for the circular amplification intermediate to be displaced downstream as rolling circle replication proceeds. If this is the case, then it follows that the extent of the amplification should be related to the extent of the deletion; very extensive amplification need not necessarily imply gross genome expansion. Another testable prediction concerning the deletion events associated with amplification is that they should lie on the side of the amplified DNA that is distal to the chromosome replication origin. The model also predicts that one end point of the deletions should lie somewhere within the unit of amplification. Precise determination of the end points of the deletions should give information concerning the mechanism by which the replication fork is reassimilated (fig.1F). For instance, if the flanking elements behave like insertion sequences, as has been suggested by several authors [16,18,19,21,28], then one end point should be the last nucleotide of the flanking element at the extremity of the amplified DNA. This sort of deletion has been observed with many transposable elements [29].

According to the proposed mechanism, the short directly repeated elements that are found associated with amplifiable DNA sequences play a cardinal role in the generation of the amplification precursor. In fact, our model predicts that any DNA sequence which contains direct repeats should have the potential to undergo amplification.

The resolution event shown in fig.1E would lead to amplification without deletion. This would cause a significant increase in the genome size (a greater than 20% increase in many cases), which might be deleterious because it would significantly increase the time required to replicate the

chromosome. It might also interfere with gross chromosome structure. There may therefore be strong selective pressure to reduce the genome size to the original value and this could be achieved by deletions occurring either adjacent to the amplified DNA or elsewhere in the genome. These considerations may afford a partial explanation for the more complex amplification events seen in certain other species, e.g. *S. reticuli* [18,30–32] and *S. glaucescens* [17,33,34].

## ACKNOWLEDGEMENT

Financial support from the SERC Biotechnology Directorate is gratefully acknowledged.

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