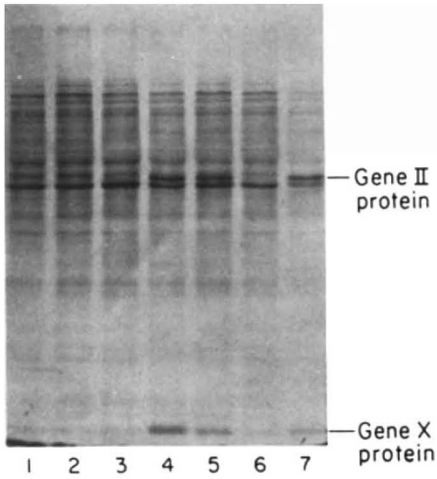

Fig. 2 Gene II protein production in cells infected with various phages. Lane 1, uninfected control; lane 2, R132 (gene II amber); lane 3, f1; lane 4, R13 (gene V amber); lane 5, R218; lane 6, Mp1; lane 7, R330. Exponentially growing *E. coli* K38 cells[21] were infected with various phages and pulse-labelled for 2 min with ^{35}S-methionine as previously described[12]. Samples were immunoprecipitated with gene II protein antiserum and analysed by electrophoresis in SDS/12.5% acrylamide gels[22,23]. The positions of the gene II and X proteins are indicated.

Using this approach, we found[12] that R218 contains a compensatory mutation (V_{218}) within its gene V coding region. The mutated gene V protein seems to retain its single-stranded DNA-binding activity, as phage is made, but loses its ability to repress translation of gene II mRNA. The resulting increased levels of gene II protein (~10-fold) inside the cell could account for the compensatory effect of V_{218} (ref. 12).

Analysis of R325 and its derivative R330 (described in Fig. 1) shows conclusively that increased gene II production is sufficient to overcome the negative effects of insertions in the phage origin. These phages, like R218, contain a mutation in their genome able to restore good growth of R218/f1. Marker-rescue experiments (Fig. 1) and nucleotide sequencing show that the mutation (II_{325}) lies upstream of gene II and consists of a single G to T substitution at position 5,977. This change lies within a sequence of the gene II mRNA leader ($U_5G_4CU_4$) which, because it seems to be unique, was suggested to be the target for translational repression by gene V protein[13]. Here we demonstrate directly the importance of this region for the regulation of gene II expression by showing that II_{325} (in R330, Fig. 2; and R325, data not shown) leads to overproduction of gene II protein in amounts similar to those observed with V_{218} (Fig. 2). Gene X protein, a product of internal initiation within gene II[14], also seems to be overproduced (Fig. 2).

Mp1 also contains a compensatory mutation which, however, does not lead to gene II protein overproduction (Fig. 2). Marker-rescue experiments (Fig. 1) show that the mutation (II_{Mp1}) lies in the gene II coding region. Nucleotide sequencing reveals a G to T substitution at position 6,125, leading to a methionine to isoleucine change (codon 40) in the gene II protein. This structural change in gene II protein has compensatory effects remarkably similar to those observed with increased gene II protein production.

Quite surprisingly, for both types of mutations, not only is the negative effect of insertions in domain B completely overcome, but domain B itself is rendered altogether dispensable. This is revealed by the analysis of chimaeric ori-plasmids containing f1 origins in which domain B is partially or totally deleted[8] (Table 1). These origins show only 1% residual biological activity relative to control (biological activity was measured as described in Table 1 legend), when f1 wild type is used to provide, in *trans*, all the necessary viral gene products. However, when the same origins are tested using R218, R330 or Mp1 as helpers, they are fully active. With Mp1 helper, mutant origins may even be more active than with the other phages.

Table 1 Biological activity of various f1 origins

Plasmids	Phages (PFU ml^{-1}) f1	R218	R330	Mp1
pD38 (A^+, B^+)	4×10^{10}	1.8×10^{10}	2×10^{10}	9×10^{8}
pD48 (A^+, B^-)	1.2×10^{12}	4×10^{10}	2.7×10^{10}	2×10^{8}
Δ+41, 70 (A^+, B^-)	2×10^{12}	5×10^{10}	4×10^{10}	6×10^{9}
Δ+29 (A^-, B^-)	3×10^{12}	1×10^{12}	2×10^{12}	1.5×10^{12}

The chimaeric plasmids used, containing the f1 functional origin or some of its parts, have been described previously[8]. A and B refer to the presence of an intact (+) or disrupted (−) domain A and B, respectively, in the various plasmids (see text and Fig. 1 legend). Phage yields were obtained as described previously[7] by infecting K38 cells that harboured the various plasmids. The ability of these plasmids to interfere with phage replication (decreased phage yields) gives a measure of the biological activity of the f1 origins that they contain[16] in the presence of the different phages. PFU, plaque-forming units.

In conclusion, we have shown that both qualitative and quantitative changes in an initiator protein can have a profound effect on the minimal sequence required for initiation of DNA replication. These findings may be of general significance and might even bear on recent, apparently contrasting, speculations concerning the role of increased production or structural alteration of certain key cellular proteins in disrupting the control of cell growth[15].

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Errata

Constraints on the neutron lifetime from triton β decay

J. Byrne

Nature **310**, 212–213 (1984)

IN the fourth paragraph on page 212, the end of the second sentence is incorrect. It should read 'as a candidate for a neutrino mass search', not 'neutron mass search'.

Unusual DNA sequences associated with the ends of yeast chromosomes

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Nature **310**, 157–160 (1984)

THE first author's name is incorrectly given as Richard W. Walmsley; the initial should be M, not W.

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