considerations may explain the large differences among five independent *FOB1* transformants that were derived from the same strain (e.g., mutant B) and had undergone the same transformation and subsequent subcultures (Fig. 4B, mutant B).

In connection with the selective advantage of cells with increased rDNA repeat numbers, we note that rDNA repeat numbers (which are still less than 10) which are attained by the limited increase through the *FOB1*-independent mechanism are not sufficient for cell growth. We found that the vector transformants of the control strain were unable to form colonies on glucose plates after 45 generations of subculture while *FOB1* transformants of control strains were able to form colonies on glucose (and to lose the helper plasmid, pNOY353). On the other hand, as emphasized previously (18), control cells with ~40 rDNA repeats had growth rates identical to those with normal (i.e., ~150) rDNA repeat numbers. Thus, expansion beyond ~40 copies appears to be achieved not because of selective advantage but presumably because of the stability of a nucleolar structure(s) carrying rDNA repeat numbers close to ~150.

In passing, we note that transformants of mutant G, which received *FOB1*, were able to expand rDNA repeats, although apparently not to the same extent as the control *FOB1* transformants. The resultant strain lacks segment G, which was originally defined as the Pol I enhancer (6), in the expanded rDNA repeats except for the single copy at the leftmost end. However, this strain was able to form colonies on glucose plates and to lose the helper plasmid. Such a strain with rDNA repeats carrying mutation G and without the helper plasmid showed only a small decrease in growth rate in glucose medium compared to the control strain with the intact enhancer in all the rDNA repeats. The role of the enhancer element in Pol I transcription is a separate subject under current study.

Relationship between rDNA repeat expansion and recombination by *HOT1*. The *HOT1* element stimulates recombination between two nearby repeat sequences at a chromosome site outside the rDNA locus. *HOT1* consists of two elements, the I element, which corresponds to the Pol I promoter, and the E element, which comprises segments F and G studied here. It has been assumed that *HOT1* activity is responsible for recombinational events within rDNA repeats. The discovery that *FOB1* is required for both *HOT1* activity (20) and rDNA repeat expansion and contraction (18) has appeared to support this assumption. However, *HOT1* activity requires active transcription by Pol I (15, 35) whereas recombinational events within rDNA repeats take place in the absence of their transcription (18). In addition, the present work has demonstrated clear differences in the *cis* elements required for stimulation of recombination between the two systems. First, segments C, D, and E are required for rDNA repeat expansion (see above) but not for *HOT1* activity (35). Second, deletion (or substitution) of segment G abolishes *HOT1* activity nearly completely (35) but reduces the extent and presumably the rate of rDNA repeat expansion only weakly (see above). (It should be noted that there is one copy of the intact G segment at the left border in mutant G used in the expansion experiments described in this paper. Thus, although we think it rather unlikely, we cannot eliminate the possibility that this single copy might play a role in recombination events responsible for repeat expansion.) The main features shared by the two systems are the requirement of segment F, which contains the RFB site, and the requirement of the intact *FOB1* gene as mentioned above. Thus, the previous assumption may be incorrect and elucidation of the mechanisms of rDNA sequence homogenization as well as rDNA repeat expansion and contraction may have to depend on the use of systems designed within the native rDNA repeat locus. In addition to the present *FOB1*-induced repeat expansion system, we have previously described experimental systems in which the effects of various factors on the expansion and contraction of rDNA repeats can be studied (18, 25). These systems should be useful in studies not only of the mechanism but also of the physiological significance of rDNA repeat expansion and contraction.

After completion of the present work, a paper by Ward et al. (37) appeared, which has demonstrated that *HOT1* activity can occur in the absence of replication fork blocking, even though both *HOT1* and RFB activities requires *FOB1*. These workers also carried out mutational analysis within the F and G segments and found that some DNA elements are shared but others are required for one activity but not for the other. Thus, their conclusion that the *FOB1* function is involved in two clearly different activities, *HOT1* and RFB activities, is related to our conclusion that it is also required for two clearly separable activities, *HOT1* and rDNA repeat expansion. Elucidation of the function(s) of the *FOB1* gene product appears to be a key to solving the intriguing problem of relationships among these three activities. In addition, consideration of these new observations made by Ward et al. (37) and by the present study raises the question whether our previous proposal is really correct, that is, whether replication fork blocking is really the first step in rDNA expansion and contraction. Although available experimental results support this proposal, they have not proven it. Detailed mutational analysis of DNA sequence elements within the F segment may be helpful to settle this question. Regardless of the answer to this question, however, the discovery of the new DNA elements that are uniquely involved in rDNA repeat expansion (and presumably also in contraction) indicates the presence of an unexplored aspect(s) of recombinational mechanisms used in rDNA repeat structures that constitute the structurally and functionally essential component of the nucleolus.

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ADDENDUM IN PROOF

We replaced the G segment, still located at the left border of rDNA repeats in mutant G. In this mutant, the *FOB1*-dependent expansion of rDNA took place as well. Therefore, the G segment was not required for the expansion.

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