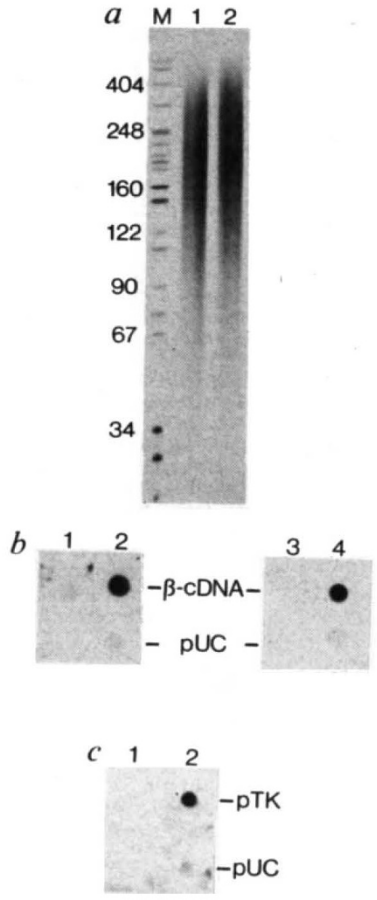

Fig. 3 Run-on assays also reveal the enhancer effect. *a*, RNA from nuclei which had been treated with RNase A and labelled *in vitro* was isolated and subjected to denaturing polyacrylamide (6%) gel electrophoresis. Cells transfected with pUβ (lane 1) and pUβE (lane 2) show a similar size distribution of *in vitro* elongated nuclear RNA; M, end-labelled marker DNA fragments (pBR322 cleaved with *Hpa*II). *b*, Unlabelled DNA of pUC9 and pcβ (rabbit β-globin cDNA) was denatured, spotted onto nitrocellulose membrane and hybridized to 4×10^6 c.p.m. (lanes 1 and 2) or 1.2×10^6 c.p.m. (lanes 3 and 4) of nuclear RNA from cells transfected with: pUβ (lane 1); pUβE (lane 2); pUβ linearized with *Hin*dIII (lane 3); pUβE linearized with *Hin*dIII (lane 4). *c*, Hybridization of pUC and pTK DNA to 6×10^6 c.p.m. of *in vitro* elongated nuclear RNA from cells transfected with pTK (lane 1) and pTKE (lane 2). HeLa cells were transfected as described previously[18]. The nuclei were isolated 36 h post-transfection as described[21], except that divalent cations were omitted from the buffers. Run-on assays were performed as published elsewhere[22].

expected to be proportional to the polymerase densities as RNase A treatment of the nuclei before *in vitro* elongation removes the pre-existing different amounts of unlabelled RNA and thus avoids competition of the mature mRNA with nascent transcripts[22]. We found that nuclear RNA from cells transfected with pUβE or pTKE hybridizes strongly to rabbit β-globin complementary DNA or *tk* DNA, respectively, whereas nuclear RNA from pUβ- or pTK-transfected cells hybridizes only weakly (Fig. 3*b*, *c*), in agreement with the S_1 nuclease mapping assays shown in Fig. 2.

The nuclear RNAs hybridize ~10% as strongly to plasmid vector DNA as β-globin or *tk* gene sequences (Fig. 3*b*, *c*), indicating (1) that the enhancer primarily stimulates transcription from the β-globin or *tk* promoter rather than from pseudopromoters within plasmid sequences, and (2) that there is efficient transcription termination preventing readthrough into plasmid DNA.

In agreement with our S_1 nuclease mapping data (Fig. 2), similar results were obtained whether transfection was performed using circular or linear DNA (Fig. 3*a*, *b*; the latter is probably ligated within the transfected cells[18]).

Thus, our results indicate strongly that an enhancer increases the RNA polymerase II density on a gene to which it is linked, that is, the enhancer facilitates transcription by increasing the frequency of initiation. In particular, the enhancer does not seem to shift the equilibrium from nonspecific to specific transcripts, as could have been inferred from the work of Humphries *et al.*[20]. These authors studied the expression of human α-, β- and δ-globin genes using vectors containing an SV40 replicon[27] and were struck by the high levels of nonspecific transcripts in the absence of an enhancer. However, we consider it likely that the high level of nonspecific β-globin transcripts is a peculiarity of their assay conditions. With replicating SV40 templates, there can be a considerable fraction of nonspecific transcripts from multiple scattered initiation points[24] which are perhaps related to the multiple initiation sites of SV40 late transcripts[28].

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Erratum

Functions of the canal system in the rotaliid foraminifer, *Heterostegina depressa*

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Nature **309**, 789-791 (1984)

ON page 789, the last sentence of the second paragraph should read "We have examined the symbiont-bearing Recent nummulitid, *H. depressa*, in which the canal system within the chamber walls and within the marginal cord replaces the primary aperture seen in other foraminifera."