bacterial nucleoid body[3]. Therefore, assuming equal concentrations of total repressor in a bacterium and in a mammalian nucleus, the fraction free to bind operator would be the same in each case. So long as the concentration of operator is low compared with the free repressor concentration, neither its absolute concentration nor the ratio of operator to nonspecific sites is relevant[3,4].

As might be expected from these considerations, the T-antigen of SV40, which binds to specific DNA sites with affinity roughly that of a prokaryotic regulator (λ repressor), is present at about the same concentration in the nucleus of a transformed cell, that bears a single T-antigen gene, as is λ repressor in the nucleoid body of a λ lysogen (R. Tjian, personal communication). Evidently T-antigen finds its operator and efficiently regulates transcription in a transformed cell[5].

Travers proposes that RNA polymerase II in eukaryotes is directed to promoter sites on DNA by protein(s) specifically bound to those sites. This idea is reasonable and consistent with emerging evidence[6,7]; my point is that it does not follow from his arguments. Indeed, were Travers correct, the auxiliary binding protein, that which binds DNA and directs polymerase to its target, would itself be unable to occupy its site efficiently.

Perhaps it is worth recalling the example of *E. coli* RNA polymerase. This enzyme binds *in vivo* to many *E. coli* promoters and initiates transcription unaided by other DNA-binding proteins. However, the enzyme efficiently recognizes other *E. coli* promoters only if guided by DNA-bound positive regulatory proteins (see, for example, refs 8, 9). The manifest function of this activator–polymerase interaction is that it affords a means of gene control—certain genes are transcribed only if the positive regulator is active, whereas other genes are transcribed constitutively, by the same polymerase.

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TRAVERS REPLIES—I recently suggested that eukaryotic RNA polymerases might locate a promoter by the initial recognition of a protein(s) prebound to this site rather than by the recognition of a specific DNA sequence[1]. This suggestion was based on two arguments: first, that in eukaryotic cells the size of the genome severely limits possible mechanisms of promoter location; and second, that the available experimental evidence suggests that DNA sequences common to most polymerase II promoters direct the binding of transcription factors other than RNA polymerase[2–4]. Ptashne suggests that the logic of my first argument is invalid.

In a seminal article Linn and Riggs[5] discussed the limitations imposed by the size of the eukaryotic genome on the equilibrium binding of a regulatory protein to its DNA target site. They showed that if the number of molecules of *lac* repressor were the same in the prokaryotic cell and the eukaryotic nucleus, a condition which is also prerequisite for my own previous discussion and conclusions[1], the repressor would be unable to occupy its binding site efficiently. They suggested four possible mechanisms which would allow eukaryotes to overcome this problem of excess DNA. These mechanisms were an increased specificity of the protein-DNA interaction, a selective masking of competing DNA, tandemly repeated binding sites or, finally, an increased concentration of the DNA binding protein.

The apparent affinity of a DNA binding protein for its target can be described by the equation[5]

$$K_{\text{eff}} = K_{\text{RO}} + D_{\text{t}} K_{\text{RO}} / K_{\text{RD}}$$

where K_{RO} and K_{RD} are the equilibrium dissociation constants of the protein for specific and nonspecific sites, respectively, and D_{t} is the total concentration of nonspecific sites. The important parameter in the present discussion is the selectivity, $K_{\text{RD}}/K_{\text{RO}}$, which determines the partition of a binding protein between specific and nonspecific sites. For the particular case of *E. coli* RNA polymerase $K_{\text{RD}}/K_{\text{RO}}$ is $\sim 10^4$ for a strong promoter[6], and $\sim 10^3$ for an average promoter. This means that in the context of a mammalian nucleus the effective number of random DNA sites would be equivalent to $\sim 6 \times 10^5$ *strong* polymerase binding sites. The number of polymerase II molecules in a mammalian nucleus is very approximately 5×10^4 (ref. 7). Clearly if polymerase II had the same value of $K_{\text{RD}}/K_{\text{RO}}$ as the bacterial enzyme and all the random DNA sites were competing with, say, 10^3–10^4 promoter sites promoter location would be kinetically inefficient and the occupancy at equilibrium would be low. To increase equilibrium occupancy one solution would be to increase $K_{\text{RD}}/K_{\text{RO}}$ as I argued previously. Alternatively a similar result could be achieved by masking at least 90% of the random binding sites. However the selectivity of the purified eukaryotic RNA polymerase is significantly less than that of the bacterial holoenzyme while a requirement for extensive masking would place constraints on the amount of DNA available for transcription at any one time. These considerations led to the suggestion[1] that promoter location would proceed more efficiently if the primary recognition of a promoter required a protein–protein interaction in place of a protein–DNA interaction.

Ptashne raises the relevant question as to how the protein which directs the polymerase to its target binds efficiently to its own DNA site. Kinetically, the problem faced by the auxiliary protein is entirely different from that for RNA polymerase. Whereas polymerase II can initiate *in vivo* at rates up to at least one every 4s the auxiliary protein–DNA complex could be extremely stable[2,4]. Consequently the polymerase needs to locate a promoter rapidly by both two- and three-dimensional diffusion processes while the auxiliary factor(s) is not constrained to the same extent by kinetic limitations and once bound to its target need only remain.

Pstashne's own comments I believe to be based on a misconception and to be irrelevant to my original discussion. His argument is dependent on two crucial premises: first, that a DNA binding protein such as the *lac* repressor would bind to its operator equally efficiently in a mammalian nucleus and in a bacterium provided that the concentration of the protein in the mammalian nucleus equalled that found in the bacterial nucleoid; and second, that the total DNA concentration (D_{t}) in the nucleus is roughly the same as in the bacterial nucleoid body[5]. The equations derived by Linn and Riggs show that the concentration of *lac* repressor required for efficient (99.9%) repression in the mammalian nucleus, assuming no DNA masking, is 2×10^{-6} M. (The value of 4×10^{-7} M quoted by Linn and Riggs assumes that 80% of the DNA is unavailable to the repressor). From this number Ptashne's premises predict, assuming 10–20 repressor molecules per cell, that the volume of the *E. coli* nucleoid is ~0.008–0.016 μm^3. The observed average volume of ~0.5–1 μm^3 (refs 9, 10) is obviously substantially greater. Thus the DNA concentration in the nucleoid must be considerably lower than Ptashne's arguments assume. Perhaps significantly, at this lower DNA concentration the calculated repressor concentration required for efficient repression corresponds approximately to the value observed *in vivo*.

Finally I particularly thank Dr R. S. Jack for many constructive discussions during the development of the model for eukaryotic promoter location.

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