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Hypothesis

Allosteric mechanism of enhancer action?

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A recurrent theme in molecular biology is 'action at a distance' along DNA. Why can some regulatory DNA sequences (enhancers) work at a great distance from the target of regulation? As one possible solution to this question we shall consider an allosteric mechanism of enhancer action focused on eukaryotic transcriptional enhancers.

Eukaryotic enhancer; Allosteric action *in trans*; Enhancer and 'promoter' element; Stable initiation complex

1. INTRODUCTION

Transcriptional enhancers are regulatory DNA sequences (elements) that are capable of activating transcription at a great distance (up to thousands of base pairs) from the promoter. A variety of enhancer action models has been proposed; at present, the 'DNA looping' model seems highly preferred [1,2]. The model assumes that enhancer-driven promoter activation is a consequence of a direct protein-protein interaction between promoter- and enhancer-bound proteins which is accompanied by 'spacer' DNA looping. The model implies that promoter- and enhancer-binding proteins have a special region (*activator* region in the case of enhancer-binding protein) to contact one another besides the DNA-binding region. It is the protein-protein interactions that provide for cooperative DNA binding because when either of two proteins is bound to DNA then the other DNA element should become relatively near to both proteins. That is, the presence of the protein at one DNA element (enhancer) increases local concentration of the second protein near its binding site. At present, this 'concentration' mechanism is generally accepted [1,3].

According to the model, any protein pair capable of cooperative binding to DNA can also interact at a distance if the protein-protein interaction is sufficiently strong. However there are many examples of cooperative binding of prokaryotic proteins and eukaryotic regulatory elements which cannot interact at a great distance although they do interact at short distances [4–6]. Hence the DNA looping model explains *how* interaction at a distance occurs but does not explain why only *some* of cooperatively binding proteins interact at a great distance. Below we discuss a modification of the model which gives a possible explanation of the action at a distance.

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2. ALLOSTERIC MECHANISM OF ENHANCER ACTION

The allosteric mechanism is shown in Fig. 1. It is assumed that both interacting DNA-binding proteins can be in two conformations: 'free' and 'DNA-bound'. The surfaces for protein-protein and DNA-protein interactions are partially 'masked' in the 'free' conformation and 'unmasked' in the 'DNA-bound' one. Such an 'unmasking' of the activator surface upon binding with enhancer has been recently shown for the yeast transcriptional activator PRTF [7].

During DNA loop formation, the 'DNA-bound' conformation is stabilized by interactions of *both* protein surfaces when in contact with DNA and another protein. Hence, the DNA-protein and protein-protein interactions are mutually strengthened thus leading to the formation of an *overall stable complex*. This 'DNA loop fixation' is a mechanism which works in the *trans* position; it depends exclusively on the strength of *induced* interactions but not on the parameters of 'spacer' DNA (length, flexibility, etc.). Note that 'independence of distance' is a key enhancer feature.

In contrast, the 'concentration' mechanism of cooperative binding is essentially DNA-length-dependent and can be described in terms of chain statistics [8]. In consequence, the mechanism works successfully only at short distances. It can be postulated that the enhancer works at a great distance (more than

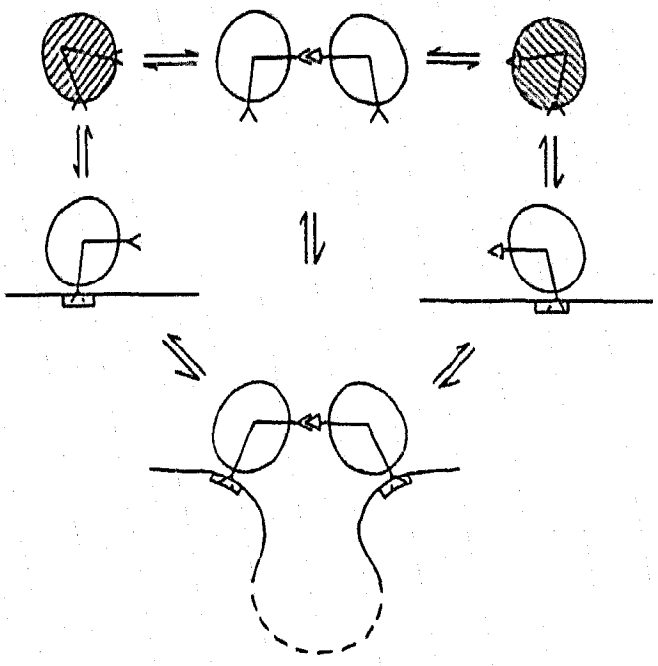

Fig. 1. Schematic representation of the allosteric mechanism of cooperative protein binding along DNA. Hatched circles and open circles correspond to the 'free' and 'DNA-bound' conformations of the interacting proteins.

200 base pairs) mainly by the allosteric mechanism. Hence the overall DNA-length dependence of enhancer action (Fig. 2) could be described as a sum of the 'concentration' and allosteric mechanisms of action.

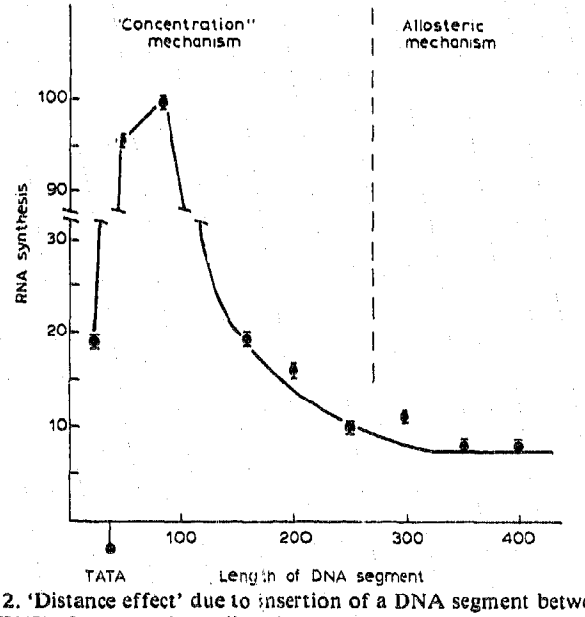

Fig. 2. 'Distance effect' due to insertion of a DNA segment between the SV40 enhancer and conalbumin promoter [20]. Relative amounts of RNA initiated at the start-site are plotted as a function of the distance between the enhancer and the promoter start-site. Proposed areas of predominant action of the concentration and allosteric mechanisms are shown.

3. EXPERIMENTAL FINDINGS

3.1. *Enhancer can be transformed into promoter element*

According to the allosteric model, the main difference between 'short-distance' and 'long-distance' transcriptional activators is not due to the different structure of the activator regions of proteins but rather to the mutual dependence of the 'unmasking' of DNA- and protein-binding regions. Therefore, if this dependence would be perturbed (for example, by protein engineering methods) then the modified activator would work only at a short distance.

A standard method for determining the activator and DNA-binding protein regions is the creation of a 'chimeric activator' [2]. Usually a significant part of the activator polypeptide chain can be deleted; modified chimeric activators work effectively even if they consist of DNA-binding and activator regions only. In agreement with the allosteric model prediction, the action of *all* chimeric activators (GAL4 derivations) tested in vivo is significantly dependent on the enhancer-promoter distance, in sharp contrast with intact GAL4 protein [9]. Some chimeric activators work more effectively than intact GAL4 but only at a short distance.

3.2. *Enhancer action* in trans

The allosteric mechanism proposes a possibility of transcription activation *in trans* after 'unmasking' of the activator region due to DNA binding. As a consequence, isolated (and hence 'unmasked') activator region can also work allosterically *in trans*. Indeed, some activator region isolated from a whole protein by protein engineering methods can work in the absence of DNA binding activity in vivo [10,11] and in vitro [12]. Since activator regions have no enzymatic activity [2], activation most probably occurs through allosteric modification of the promoter-binding protein.

It has also been demonstrated that allosteric effects participate in the action of natural activators. Enhancer elements were introduced in the cells in the form of a short synthetic double-stranded DNA fragment [13]. The latter effectively interacted with enhancer-binding proteins in vivo and *increased* the promoter activity over the level provided by endogenous enhancer. The most probable interpretation is that binding of a synthetic enhancer leads to 'unmasking' of an activator region which induces the promoter through protein–protein interaction (by analogy with isolated activator regions) [13]. If this interpretation is correct, then DNA-dependent 'unmasking' of contact surfaces occurs in both proteins interacting with the promoter and the enhancer.

It should be pointed out that the 'concentration' mechanism also suggests a possibility of enhancer action *in trans* but only if the enhancer and promoter elements are in close proximity before activation (as,

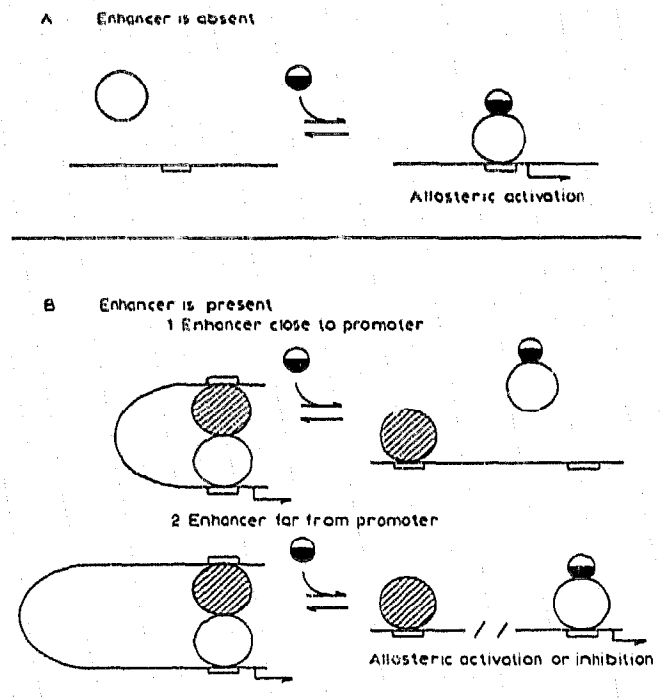

Fig. 3. Possible mechanisms of *in trans* activation and inhibition (squelching) of eukaryotic promoters by isolated activator polypeptide in the absence (A) and in the presence (B) of endogenous enhancer. Open and hatched circles correspond to the promoter- and enhancer-binding proteins. The small circle represents the isolated activator polypeptide.

for example, in catenans where enhancer- and promoter-containing plasmids are interlinked [14]). Therefore the above examples of enhancer action *in trans* cannot be explained in the framework of the 'concentration' mechanism.

Another example of enhancer action *in trans* is a 'squelching' effect [15]: it was shown that the enhancer-binding protein (or isolated activator region) can negatively regulate transcription initiation in vivo if added to another promoter-enhancer system. Therefore enhancer-binding proteins can activate as well as suppress transcription *in trans*. What is the reason for this paradoxical effect?

One possible explanation follows directly from the allosteric mechanism (Fig. 3). What are the consequences of the addition of an activator polypeptide to an enhancer-promoter system? Apparently, if the enhancer is absent then only allosteric activation is possible (Fig. 3A). If the enhancer is present, then the activator polypeptide interaction with the promoter protein can lead to disruption of the promoter-enhancer DNA loop (Fig. 3B). As a result, the overall effect can be either negative ('concentration' squelching), or positive, depending on the architecture of the regulatory region and the concentration of the proteins in the system tested.

3.3. *Enhancers and stable initiation complexes*

The allosteric model predicts that enhancer-driven transcriptional activation leads to the formation of overall stable initiation complexes including both enhancer and promoter. Indeed, a high concentration of competing enhancer sequence causes removal of proteins from the *working* enhancer neither in vivo [16] nor in vitro [17,18]. In contrast, if a competing enhancer is present *before* initiation of transcription then activation is suppressed. The data have been interpreted previously in the framework of the 'hit-and-run' model but the model has recently been disproved [19]. The recent data on the occurrence of an overall stable complex in the case of the ribosomal enhancer [14] also suggest that its formation can be realized by the allosteric mechanism.

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