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MINIREVIEW

Distant Activation of Transcription: Mechanisms of Enhancer Action

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Enhancers are regulatory DNA sequences that activate transcription over long distances. Recent studies revealed a widespread role of distant activation in eukaryotic gene regulation and in development of various human diseases, including cancer. Genomic and gene-targeted studies of enhancer action revealed novel mechanisms of transcriptional activation over a distance. They include formation of stable, inactive DNA-protein complexes at the enhancer and target promoter before activation, facilitated distant communication by looping of the spacer chromatin-covered DNA, and promoter activation by mechanisms that are different from classic recruiting. These studies suggest the similarity between the looping mechanisms involved in enhancer action on DNA in bacteria and in chromatin of higher organisms.

Enhancers (Es) are short (20- to 400-bp) DNA sequences that can activate transcription from target promoters (P) in *trans* and over various distances (more than 100 kb) (7). Enhancers operate in pro- and eukaryotes; in the majority of cases, action of Es involves direct E-P interaction through proteins bound at the E and P, accompanied by formation of an intervening chromatin loop (7, 24, 38). Recent genomic studies using various versions of the 3C approach revealed widespread use of gene regulation by enhancers (see reference 32 for a review). In parallel, genomic studies identified specific signatures (histone modifications and associated proteins) of enhancers that greatly facilitated analysis of the databases (32).

At the same time, understanding of mechanistic aspects of enhancer action trails behind, primarily due to the lack of *in vitro* systems faithfully recapitulating distant activation. The enhancer field remains driven by the concept of recruiting that was proposed to explain short-distance activation of transcription in prokaryotes (Fig. 1A) (44). During recruiting, an activator protein increases the local concentration of another protein/protein complex (e.g., RNA polymerase [RNAP]) in the vicinity of its binding site. The local increase of protein concentration results in relief of a step limiting the rate of initiation (usually binding of RNAP to a promoter nearby) and induces transcription. During distant action, even if a protein complex was recruited to the enhancer, its concentration at the target would not necessarily be increased because E/P do not typically colocalize. Furthermore, enhancers typically activate preformed complexes already recruited to DNA (Fig. 1B; also see below). Thus, the concept of recruiting cannot explain some principal aspects of enhancer action; instead, the presence of preformed enhancer targets raises questions about efficient E-P communication and activation of transcription (Fig. 1B). In this review, we focus primarily on mechanistic aspects of enhancer action; other recent studies were covered in several excellent reviews (7, 24, 32, 38).

ENHANCER ACTION ON DNA

In prokaryotes, there are two types of transcriptional enhancers using tracking and looping mechanisms for enhancer-promoter communication (EPC) (7). Only the looping mechanism is shared between pro- and eukaryotic enhancers and is considered here. In *Escherichia coli*, the looping mechanism is employed by NtrC-dependent transcriptional enhancers activating σ^{54}-dependent promoters; it has been extensively studied using the *glnAp2* promoter as a model (see reference 5 for a review). NtrC is an activator protein complex that binds to the enhancer, and, after phosphorylation and oligomerization, it interacts with the Eσ^{54} RNAP (bound as a stable, inactive, closed complex at the target promoters), and it also activates conversion of the inactive complex into the productive open initiation complex. As the RNAP leaves the promoter, the σ^{54} subunit dissociates. During the transient enhancer-promoter interaction, the intervening DNA is looped out.

While the concept of DNA looping explains how enhancer- and promoter-bound proteins interact, it does not automatically explain the high efficiency of E-P communication. In fact, DNA sequences separated by more than 1 kb do not communicate efficiently on linear DNA *in vitro* (4); therefore, enhancer action over a long distance requires use of special facilitating mechanisms (30). Thus, a short distance is typically <1 kb; efficient EPC within this range does not necessarily require special facilitating mechanisms.

Computer modeling studies suggest that the average distance between linearly separated DNA regions could be considerably decreased on supercoiled DNA (53). Indeed, DNA supercoiling greatly facilitates EPC over a long, but not over a short (<1 kb), distance through a mechanism that involves a global change of DNA conformation (5, 30). Computer simulations and experimental studies suggest that DNA supercoiling results in formation of DNA branches and thus increases the probability of juxtaposition between linearly separated DNA sites (Fig. 2) (17, 43), provided that DNA branches are dynamic structures. Indeed, it has been computationally predicted (17) and experimentally established (6) that communication within each DNA branch occurs by slithering (fast movement of intertwined DNA helices within and along the DNA branches on supercoiled DNA) (Fig. 2). The slithering model, in contrast to the more traditional tracking model of

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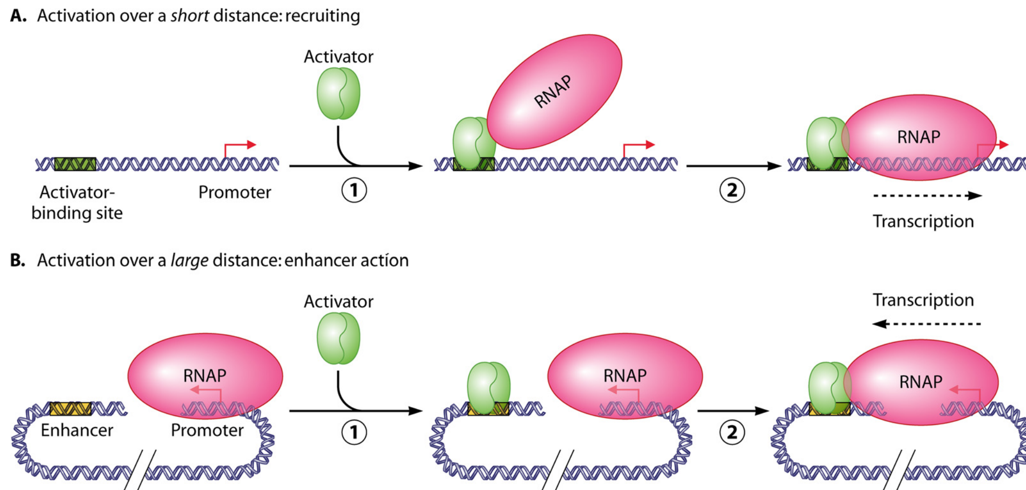

FIG 1 Mechanisms of transcriptional activation over short and long distances: recruiting versus DNA looping. (A) Recruiting. As an activator binds to DNA (step 1), it recruits (increases the local concentration of) another protein (e.g., RNA polymerase), the binding of which is a rate-limiting step during transcription initiation (step 2). (B) Enhancer action. Binding of an activator (or a recruited protein, not shown) to the enhancer (step 1) does not automatically increase its local concentration at the target promoter because the promoter and enhancer do not colocalize. Thus, enhancer-bound protein has to (i) efficiently explore surrounding DNA/chromatin regions, (ii) identify the target promoter (e.g., marked by RNAP), and (iii) interact with and activate the promoter by a mechanism that is different from recruiting (step 2). This interaction is typically accompanied by looping of the spacer DNA or chromatin.

enhancer action (see reference 7 for a review), does not require directional movement of the enhancer toward the promoter or involvement of a special protein moving along DNA.

To experimentally evaluate the contribution of this mechanism to the high rate of E-P communication on negatively supercoiled DNA, slithering was prevented by introducing two *lac* operators surrounding the *glnAp2* promoter (Fig. 2) (6). The *lac* repressor formed a protein bridge that placed the promoter and

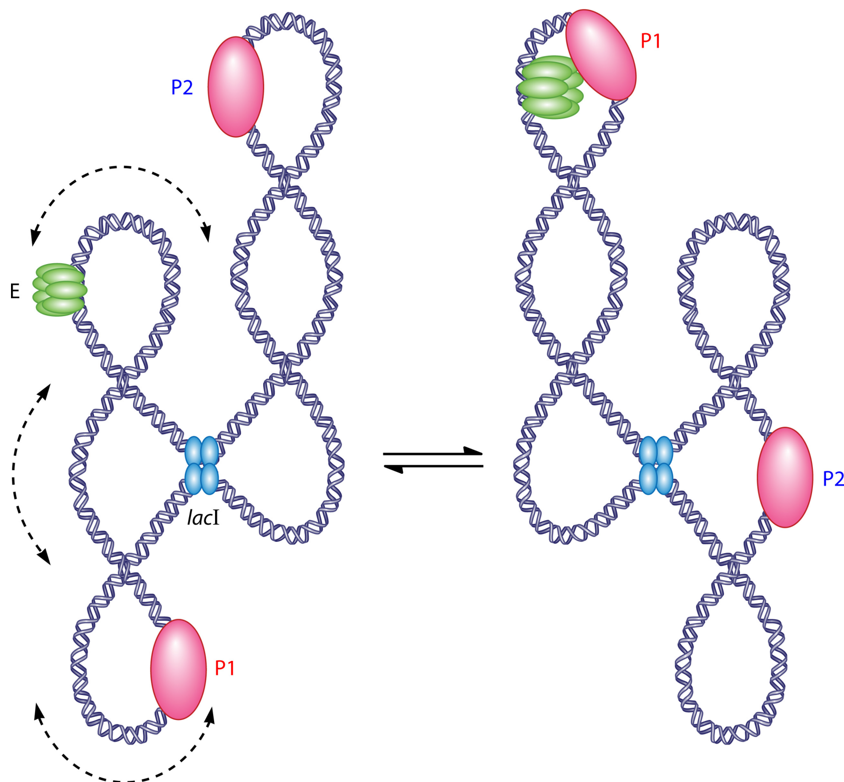

FIG 2 Slithering mechanism of facilitated distant communication on DNA (6). Two identical promoters (P1 and P2) are differently positioned relative to an enhancer (E) on a plasmid and are separated by a protein bridge (insulator) formed by the *lac* repressor (*lac*I). Sliding of intertwined DNA helices within branches formed on supercoiled DNA (slithering; dashed arrows) greatly increases the probability of E juxtaposition with the promoter P1 positioned within the same topological DNA domain (6, 43). In contrast, the P2 promoter positioned on a different domain of the same DNA molecule cannot efficiently communicate with the E because slithering through the protein bridge is impossible.

the enhancer on topologically isolated DNA loops and prevented DNA slithering between the loops. The presence of the *lac* repressor results in selective and strong inhibition of the promoter positioned on the topologically isolated DNA loop; an identical promoter positioned within the same loop with the enhancer remains fully active, suggesting that EPC within each DNA loop is permitted. The data support the slithering model of enhancer action and indicate that the *lac* repressor can strongly inhibit transcription over a long distance (>1.5 kb from the promoter) via formation of DNA loops that topologically isolate the promoter and enhancer. These features make *lac* operators formally similar to eukaryotic insulators blocking enhancer action over a distance. Further experiments revealed that the protein bridge assayed in the prokaryotic system *in vitro* has all of the enhancer-blocking properties of eukaryotic insulators described *in vivo* (6).

In summary, analysis of NtrC-dependent enhancer action revealed several principles that are often used during action over a distance (see below), including the following: (i) formation of a stable, inactive protein-DNA complex at the enhancer and target promoter before activation; (ii) use of special mechanisms facilitating distant communication; (iii) promoter activation via a mechanism that is different from that of recruiting; and (iv) reestablishing the looping after each activation event.

ENHANCER ACTION IN CHROMATIN

Prokaryotic enhancers typically work over distances of up to several kb (7), while eukaryotic enhancers often communicate over a >100-kb range and in *trans* (28, 33). Additional challenges faced by eukaryotic enhancers include constant competition between binding of sequence-specific proteins and chromatin formation at the enhancers and promoters and the need to communicate over chromatin-covered DNA. The molecular mechanisms of action of eukaryotic enhancers are not well studied, primarily because of the limitations of the *in vitro* systems poorly supporting distant enhancer action (3, 26, 47).

Inactive chromatin in interphase eukaryotic cell nuclei is in a highly condensed state and is localized within distinct chromosome territories (12). In order to be activated, a gene has to be relocated outside the chromosome territory and its chromatin structure decondensed to a level consistent with the existence of the 30-nm chromatin fiber (35). Poised (potentially active in transcription) and active chromatin domains likely exist in the form of the 30-nm fibers (13 and 37; but also see reference 51). Thus, the spacer DNA separating communicating eukaryotic enhancers and promoters is likely organized into the 30-nm chromatin fiber.

As in prokaryotes, the inactive enhancer and the target promoter are preset before activation. In this case, potentially active promoters are marked by the presence of specific patterns of histone methylation (H3K4me3 and H3K27me3), histone variants H3.3 and H2A.Z, and elongating (paused) RNA polymerase II (Pol II) making short transcripts (15, 18, 22, 31). Elongating Pol II is one of the most stable DNA-protein complexes in eukaryotic nuclei and likely serves two functions: it protects poised promoters against formation of inactive chromatin structure (14) and constitutes a highly stable target for enhancer action. Poised enhancers are marked by H3K4me1/2, H3.3, and H2A.Z, as well as by formation of specific, functionally inactive DNA-protein complexes (different for different enhancers) (22, 31, 50). During cell differentiation these inactive, stable complexes are often replaced by different, tissue-specific DNA-protein complexes that then activate the target promoters (see reference 39 for a review). Active enhancers are specifically marked by H3K27ac (45).

Similar to the NtrC-dependent enhancers, all well-studied eukaryotic enhancers work by looping: in the active state the enhancer and target promoter are in physical proximity (see references 7, 24, and 38 for reviews). How can chromatin looping occur efficiently? The simplest, most straightforward model suggests that chromatin-compacted DNA supports efficient EPC. Functionally active genomic regions usually contain E-P spacer DNA that is compacted up to ~30-fold into the 30-nm chromatin fiber (35). Therefore, the range of action of the recruiting mechanism could be increased up to 30-fold (to ~20- to 30-kb range), provided that the chromatin structure is dynamic. Indeed, recent studies suggest that chromatin structure maintains efficient EPC *in vivo* (46). Furthermore, chromatin structure can greatly facilitate intramolecular ligation of distantly spaced DNA ends (49) and support efficient distant EPC *in vitro* by the looping mechanism (26, 47). The high efficiency of EPC cannot be explained only by chromatin-induced DNA compaction (25, 47). Interestingly, DNA supercoiling does not affect the high rate of EPC in chromatin, suggesting that chromatin structure *per se* can support highly efficient communication over a distance and functionally mimic the supercoiled state characteristic for prokaryotic DNA (47). To explain the efficient communication in chromatin, it has been proposed that spontaneous uncoiling of the ends of nucleosomal DNA from the histones provide dynamic DNA flexibility that facilitates distant EPC (Fig. 3A) (47).

Further studies have suggested that the mechanisms of communication on supercoiled DNA and in chromatin are even more similar. Thus, the protein bridge that blocks distant action of bacterial enhancers *in vitro* (Fig. 2) has all the enhancer-blocking properties of eukaryotic insulators described *in vivo* (6, 55). Furthermore, a very similar protein bridge formed using the tetracycline-controlled ttA-TetO system has a strong enhancer-blocking insulator activity in transiently transfected mammalian cells (2), suggesting that formation of a chromatin loop topologically isolating the enhancer from the target promoter is sufficient to block EPC *in vivo*. These experiments suggest that similar mechanistic principles could be utilized during EPC in eukaryotic chromatin and on supercoiled DNA of prokaryotes. Recent data obtained using assembled chromatin arrays suggest that internucleosomal interactions involving the histone tails are essential for highly efficient, long-range EPC in chromatin *in vitro* (25). We speculate that long-range, transient internucleosomal interactions through the histone N-terminal tails (16, 21) keep chromatin fibers in close proximity and allow relocation of the interacting fibers relative to one another (Fig. 3B). These relocations could occur by a mechanism involving sliding of interacting chromatin fibers (termed brachiation; Fig. 3B, mechanism 1) or by reestablishing internucleosomal interactions *de novo* (Fig. 3B, mechanism 2) (36).

At the same time, recent data suggest that chromatin structure *per se* is not sufficient for distant EPC, and some additional facilitating mechanisms are used in higher organisms. Genomic studies show that many enhancers and the spacer DNA are transcribed, producing noncoding RNA (ncRNA) molecules with an average size of 1 kb (23, 40); the transcripts and/or ongoing transcription are required for enhancer action (23, 40, 54, 57). Two models have been proposed to explain these observations. For some enhancers, the enhancer DNA together with Pol II and TATA binding protein (TBP) tracks along the intervening DNA

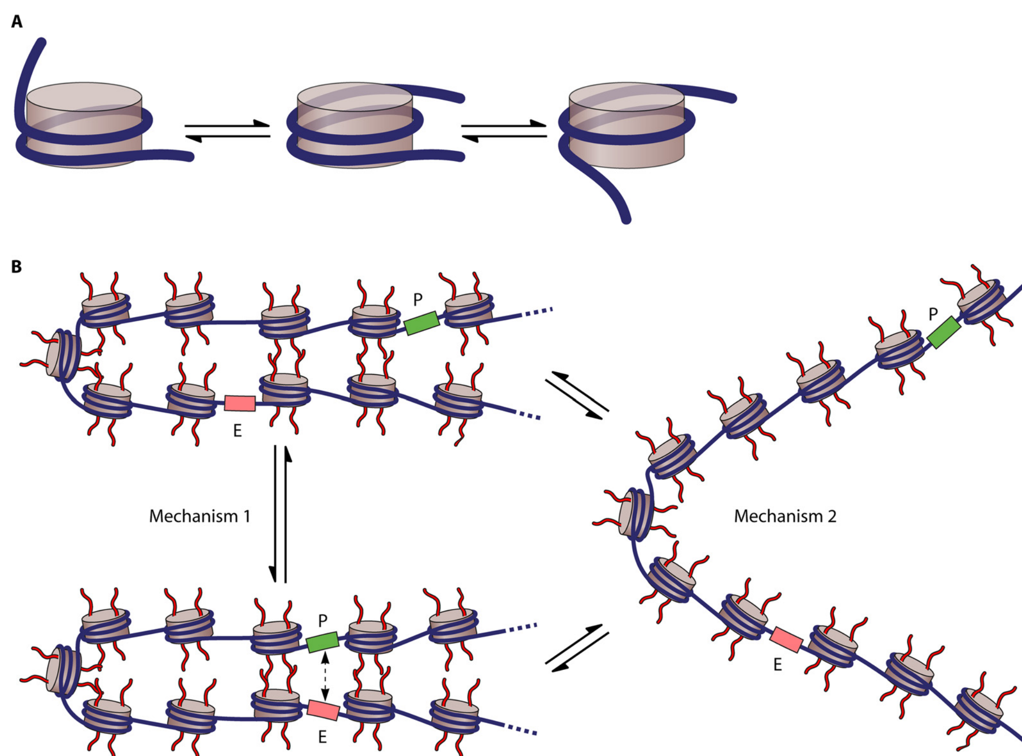

FIG 3 Proposed mechanisms of highly efficient EPC in chromatin. (A) DNA uncoiling mechanism. Partial spontaneous uncoiling of the ends of nucleosomal DNA from the octamer occurs at a high rate (27, 42). Thus, each nucleosome in an array can provide two points of dynamic histone-induced DNA flexibility at the positions where DNA enters and exits nucleosomes. (B) The hypothetical mechanisms of long-range EPC involving histone N-tails. In mechanism 1, termed brachiation, transient internucleosomal interactions mediated by histone tails could keep chromatin arrays or fibers in close proximity and allow relocation of the interacting partners relative to each other at a high rate. In mechanism 2, termed transient collapse, multiple intrafiber interactions mediated by histone tails could be fully disrupted and reestablished in a different register (36).

(57), presumably forming unstable chromatin loops before reaching the promoter and forming the activation loop. In this case, the process of transcription itself is essential, since a terminator or an insulator inserted between the enhancer and the promoter traps Pol II and blocks long-range enhancer function (29, 57). Since Pol II movement is not highly processive, the putative intermediate loops may need to be stabilized. At the same time, genomic studies have revealed that the presence of ncRNA *per se* is required for enhancer action (23, 40, 54), suggesting a second model where RNA stabilizes the E-P chromatin loop, either during or after communication. It is also possible that ncRNA transcription locally affects chromatin structure or the pattern of histone modifications.

After formation of the activation chromatin loop, it is likely stabilized by additional protein factors, such as CTCF and cohesin (see references 10, 11, and 56 for reviews). CTCF is a sequence-specific insulator-binding protein, and cohesin can form large complexes that could incorporate two DNA molecules and thus stabilize the chromatin loop. In some cases, regulatory E-P chromatin loops are formed with the mediator and cohesin (20). Formation of chromatin loops can occur in *cis* and in *trans*; in fact, looping interactions only rarely occur with the nearest gene (48). It is unclear whether the chromatin loop has to be reestablished after each round of transcription, as it occurs in prokaryotes. It is tempting to propose that EPC over a long distance in higher organisms requires special mechanisms preventing complete disruption of the loops after each round of transcription.

Like in bacteria, activation in eukaryotes occurs via mechanisms that are different from those of classic recruiting. Two mechanisms of enhancer action have been studied in detail. Transcriptional activation facilitating formation of the preinitiation complex involves recruiting of transcription factor IID (TFIID) on the promoter and its activation by TFIIA (8). TFIIA-TFIID interaction results in a change of TFIID conformation leading to a more stable interaction with the promoter and formation of functionally active, committed preinitiation complex (41). More recently, genomic studies have revealed that many enhancer-activated genes contain paused Pol II (9, 14, 15, 34) that most likely protect these poised promoters from inactivation by competing chromatin assembly (14). Since elongating Pol II complex is one of the most stable DNA-protein complexes in the nuclei, paused Pol II provides a very stable target for enhancer action. Some poised genes also contain stably bound, paused Pol II (19). Thus, in all studied cases, poised promoters contain stably bound DNA-protein complexes and are enhancer activated by the mechanisms that are different from classic recruiting.

CONCLUSIONS

In summary, recent studies identified striking mechanistic similarities between the bacterial and eukaryotic enhancers, revealing fundamental principles of action over a distance. In the majority of cases, inactive, stable protein-DNA complexes are formed at the enhancer and target promoter before activation. This indicates that enhancers are unable to establish a promoter *de novo*, since in

this case it is unclear how the target promoter is identified. On activation, EPC is accompanied by chromatin looping and involves use of special mechanisms facilitating distant communication. Promoter activation occurs via a mechanism that is different from recruiting, likely because recruiting does not work over long distances.

The widespread use of enhancers in gene regulation dictates their involvement in development of human diseases (see reference 52 for a review). Indeed, recent studies revealed thousands of variant enhancer loci (VEL) that comprise a signature that is predictive of colon cancer (1). Without doubt, future studies of enhancers will reveal new mechanisms of development of human diseases and provide new targets for their treatment.

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