The Establishment of Active Promoters in Chromatin

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Summary

The organization of eukaryotic genomes as chromatin provides the framework within which regulated transcription occurs in the nucleus. The association of DNA with chromatin proteins required to package the genome into the nucleus is, in general, inhibitory to transcription, and therefore provides opportunities for regulated transcriptional activation. Granting access to the *cis*-acting elements in DNA, a prerequisite for any further action of the *trans*-acting factors involved, requires the establishment of local heterogeneity of chromatin and, in some cases, extensive remodeling of nucleosomal structures. Challenging problems relate to the establishment of this heterogeneity at the level of the single nucleosome and to the mechanisms that operate when nucleosomal arrays are reorganized. Recent developments indicate that chromatin reconstitution in cell-free systems allows the biochemical analysis of the interplay between transcription factors and chromatin components that brings about regulated transcription.

Introduction

The frequency with which journal editors request reviews on a particular topic indicates both the general interest in the subject and the rapidity of the developments within the field. Judging from the number of excellent recent reviews that have explained the current status of our knowledge on chromatin structure or furthered our understanding of how chromatin is involved in transcriptional regulation[1-11], much progress has been made over the last few years. In this article we would like to continue and deepen a topic that has just been dealt with in these columns[2], namely how active promoters are established in chromatin. Our special emphasis here will be to summarize the most recent work that may indicate what the mechanisms of chromatin reorganization by transcription factors at the level of a single nucleosome might be.

Repressive higher order structures must be resolved

Chromatin is organized in a hierarchy of structures, from the basic repeat unit, i.e. the winding of the DNA around the histone octamer to form nucleosomes, to the complex appearance of metaphase chromosomes. Each level of chromatin organization contributes to the dense packaging of DNA, effectively repressing gene expression. Single positioned nucleosomes and arrays of regularly spaced nucleosomes can be reconstituted in cell-free systems, and in these systems the lower levels of structural organization and their functional implications are amenable to detailed analysis. The complex folding of the nucleosomal fiber, collectively termed 'higher order structures', cannot be reconstituted in cell-free systems and therefore our understanding of those structures is still diffuse. Highly compacted and transcriptionally inert heterochromatic regions, such as those found in centromeres, serve as microscopically visible examples. The confinement of heterochromatin to specific regions on chromosomes is the result of an interplay between a multitude of gene products that have been genetically characterized in *Drosophila* as enhancers and suppressors of 'Position Effect Variegation' (PEV)[12,13]. The phenomenon of PEV illustrates the dominant repressive effect of presumed higher order structures on otherwise active genes that suffer from translocation into the proximity of heterochromatin. In certain mutant flies, heterochromatin tends to 'spread' from its normal location into neighboring euchromatin, where it suppresses the activity of affected genes. The class of proteins whose elimination enhances the spreading of heterochromatin and PEV includes transcription factors (see below), while mutation in proteins thought to structurally define heterochromatin, such as HP1, an abundant heterochromatin-associated protein, suppresses PEV (reviewed in ref. 14). Sequence similarity between HP1 and *Polycomb* (reviewed in ref. 15), a member of a group of genes involved in maintaining the repressed status of homeotic genes in the context of the appropriate developmental program, suggested the idea that a higher order chromatin structure may be involved in regulating devlopmental processes in the fly. Mapping of *Polycomb* binding sites after crosslinking in intact cells revealed an excellent correlation between the repressed homeotic gene loci and *Polycomb* association[16]: while *Polycomb* was bound to the chromatin of the transcriptionally repressed *Ultrabithorax* and *abdominal-A* genes, it was not detected in the vicinity of the active *Abdominal-B* gene. The presence of *Polycomb* (and other associated members of the family) at many loci in the genome[17], in chromatin domains that may extend for 100 kb, again argues that chromatin folding has a general role in regulating gene activity during development. The question remains as to how the repressed domains are defined. As Orlando and Paro[16] discuss, repressors binding to the genetically defined silencer elements located within the domains could be the starting points for the spreading of an inert structure throughout a domain. An analogous mechanism could be at work during silencing of the yeast mating type loci where, apparently, the targeting of SIR1 protein to the silent cassettes through specific interactions with sequence-specific DNA binding proteins can initiate silencing[18]. Unfortunately, we know even less about how the formation of higher order structures can be reversed or prevented to allow for activation of promoters at the level of the nucleosomal DNA. One of the burning questions is, whether the resolution of repressive superstructures requires modifications at

this very level, or whether changes within the underlying level of nucleosomal DNA would directly translate into unfolding. Answering this question is complicated by the possibility that the assumed modifications at the nucleosomal level might not necessarily mark the domain throughout, but could be restricted to strategic 'triggering' positions that could, for example, coincide with the silencing elements in the domains governed by *Polycomb* regulation. While this possibility clearly exists, it is nevertheless also worthwhile considering the alternative mechanism that the general modification status of nucleosomal DNA determines the tightness of higher order packaging.

Specific histone acetylation patterns characterize active chromatin domains

A striking example of how regional heterogeneity of nucleosomes correlates with gene activity and higher order structure comes from studies on the acetylation pattern of core histone N-terminal tails that protrude from the nucleosomal particle[19]. Turner and colleagues[20] have used a set of antibodies able to distinguish between acetylated histone H4 isoforms to probe for the presence of acetylations at defined N-terminal lysines in *Drosophila* polytene chromosomes. They showed that while acetylation at H4 lysines 5 and 8 occurred in distinct, nonidentical patterns in euchromatic regions, acetylation at lysine 16 was predominantly associated with chromatin of the transcriptionally hyperactive male X chromosome. The latter acetylation pattern appears to be tightly connected to the mechanisms that control dosage compensation in the fly, and correlates perfectly with the binding of the *maleless (mle)* and *male-specific lethal (msl)* proteins to the same regions[21]. Just as *Polycomb* and associated factors are bound to repressed chromatin domains over their entire length, *mle* and *msl* proteins mark the hyperactive chromatin in the male X chromosome. In mammals, where dosage compensation is achieved by inactivation of one X chromosome in females, acetylations are absent on the inert, heterochromatic X chromosome[22]. Similarily, hypoacetylation of core histones is observed in silenced mating type cassettes and telomeres in yeast[23]. Genetic analyses in *Drosophila*[24] and yeast[23] of factors responsible for heterochromatin formation and silencing have enabled identification of factors that promote histone deacetylation. The puzzling fact that, in the fly, nucleosomes in the heterochromatic chromocenter are enriched in H4 isoforms acetylated at lysine 12[20], suggests that the rules relating modification patterns at the nucleosomal level to higher order structure may be complex. It is, however, worth speculating that histone modifications that paint large chromatin domains and even whole chromosomes, may nevertheless be important for local phenomena at promoters, together with the sequence-specific action of transcription factors (see below).

Local accessibility at active promoters

The presence of higher order structures dominates the potential of a promoter to be actively transcribed. While a permissive environment is clearly a prerequisite for any further steps, the resolution of superstructures does not automatically lead to transcription. Because many transcription factors cannot interact with their binding sites on a nucleosome (reviewed in refs 5, 8), which is the lowest level of chromatin organisation, the nucleosomal fiber, with or without associated linker histone, will still repress basal transcription. Correspondingly, active promoters and enhancers are usually characterized by a special chromatin organisation with increased accessibility. This open configuration can be visualized by its increased sensitivity towards DNase I digestion in whole nuclei. The operational definition of active sites as DNase I-hypersensitive does not tell much about the precise nature of these sites, but it seems clear at least that they do not generally contain classical nucleosomes. This view has recently been challenged by McPherson et al.[25], who reported the apparent nucleosomal nature of an active enhancer that displayed transcription factors on the surface of a nucleosome. This type of scenario would certainly expand the options for promoter structures in chromatin; however, given the ambiguities of nucleosome mapping by micrococcal nuclease digestion[26], other interpretations of the data remain.

The mechanisms by which a special class of transcription factors create access at promoters have been classified as either 'pre-emptive' or 'dynamic' competition with nucleosomes[9], depending on whether the establishment of nucleosome-free regions requires the general chromatin perturbation that accompanies replication, or whether nucleosome disruption occurs in direct response to the activation of a crucial transcription factor in the absence of replication. Accordingly, the promoters have been classified as either 'preset' or 'requiring remodeling'[2] or 'persistent' and 'inducible' nucleosome-free[5]. The latter class of chromatin remodeling events, which occur in chromatin of promoters that are activated in response to sudden environmental stimuli, has been described in detail[6,9,10]. Recent efforts have been directed towards unraveling the mechanisms underlying nucleosome remodeling by developing *in vitro* reconstitution systems. We will discuss the progress made in three such systems, namely nucleosome disruption by the yeast GAL4 protein, the *Drosophila* GAGA factor and the mammalian glucocorticoid receptor.

Nucleosome destabilization by GAL4 requires concerted action with accessory molecules

Induction of the GAL1 promoter in yeast results in an increased accessibility of the TATA box in chromatin, which depends on the action of the crucial transcription activator GAL4[26]. In a model system, Morse[27] has demonstrated the ability of GAL4 to disrupt a positioned nucleosome containing a single GAL4 binding site near its centre in the yeast cell. Similar conclusions have been reached using *in vivo* photofootprinting by Axelrod and coworkers[28], who also demonstrated a requirement for the acidic activation domain of GAL4 and correlated the efficiency of nucleosome disruption with the apparent strength of the activation domains of GAL4 derivatives. The unprecedented power of the artificial activator protein GAL4-VP16, which is made up of the

GAL4 nucleosome binding domain and one of the most powerful acidic transcription activation domains from herpes simplex virus, extends this correlation, although it is presently unclear whether nucleosome disruption (anti-repression) and transcriptional activation are brought about by the same features of the domain. GAL4-VP16 will relieve transcriptional repression by replication-coupled chromatin assembly in *Xenopus* oocytes[29] and counteract chromatin repression during early embryonic development[30].

There is considerable evidence that GAL4-VP16 is able to disrupt nucleosomes *in vitro*, too. Nucleosome disruption was deduced from the anti-repressive effect of the activator when nucleosomal templates were transcribed in extracts from yeast[31] or from *Drosophila*[32]. Kamakaka et al.[32] found that GAL4-VP16 could activate transcription from templates containing nucleosomal arrays with regular spacing, but not if these nucleosomes were stabilised by incorporation of the linker histone H1, an interesting result in the context of the finding of Workman and coworkers[33], namely that GAL4 derivatives bind equally well to multiple or to single sites on nucleosomes that do or do not contain histone H1. The significance of this observation for the regulation of transcription by the GAL4 protein remains unclear, since to date no H1-homologue has been identified in yeast.

The GAL4 protein and its derivatives are members of a special class of transcription factors that can interact with their binding sites under the adverse steric and conformational conditions presented by DNA in a nucleosome[8]. This interaction is not without problems, but is greatly facilitated if the binding sites are closer to the end of the nucleosome, where DNA is less tightly associated with the histones[34]. Multiple binding sites on the surface of the particle will allow cooperative invasion of the nucleosome by GAL4 molecules, starting from the edges, and even sites near the center of the nucleosome will be filled eventually. The uncooperative binding of GAL4 to a single element near the center of the nucleosome is poor, but greatly stimulated by removal of the N-terminal tails of the core histones[34], which are thought to interact with DNA on the surface of the nucleosome and in the linker. In a similar type of experiment, the binding of TFIIIA to a nucleosomal target was also facilitated when histone tails were removed, indicating that the tails are major determinants of site accessibility in nucleosomal DNA[35]. In the latter study, acetylation of the N-terminal lysines had a very similar effect to that of the deletion of the tails, supporting the idea that changing the properties of the histone tails by neutralizing positive charges in the nucleus will modulate the access of regulators to nucleosomal DNA. The above-mentioned localized acetylation at specific H4 lysines in distinct chromatin domains may thus not only reflect requirements for untangling higher order chromatin structures, but may also have implications for factor access at the level of the single nucleosome. In the case of GAL4 regulation, the situation is not that straightforward. Deletions and substitutions of amino acids in the N-terminal tail of histone H4 in yeast result in a much reduced transcriptional stimulation of the GAL1 promoter, indicating that H4 tails not only need to be stripped off the DNA, but also that they are targets of an interacting factor involved in overcoming nucleosomal inhibition[36]. By contrast, substitution of lysines in the N terminus of H3 by other amino acids resulted in increased activation of this promoter, suggesting that complex functions are associated with individual amino acids in certain histone tails[37]. These and related data suggest that the mechanisms by which nucleosomes are destabilized may involve a triggering by direct interaction of transcription factors with the protruding histone tails, an effect that has not been reproduced in a cell-free system yet.

There are indications that even the potent GAL4-VP16 cannot overcome nucleosomal inhibition on its own. While addition of the activator to a crude yeast transcription system relieved transcriptional inhibition of a chromatin template, presumably by nucleosome destabilization, GAL4-VP16 was not able to activate a chromatin template in the context of a purified transcription system consisting only of basal transcription factors and polymerase[31], and apparently required a biochemically distinct mediator. Similar observations were made using a *Drosophila* transcription system[38]; however in this case the auxiliary factor turned out to be RNA, which may have functioned as a histone sink. Kadonaga and coworkers[32] reported that, in order to anti-repress a chromatin template that contained H1, GAL4-VP16 had to be present during the chromatin assembly reaction. Hence GAL4-VP16 acted in the presence of the crude assembly extract that could have been the source of auxiliary molecules, which would be absent if the potential of the factor was tested after replication, i.e. on a purified chromatin template.

It is likely that upon complete disassembly the histones will need to be sequestered by some kind of carrier molecule. Using a model system of low complexity, Workman and colleagues have suggested another idea about the nature of this histone acceptor[39]. If nucleoplasmin, a histone carrier with preference for H2A/H2B and known to catalyze the assembly of nucleosomes from histones[40,41], was added along with a GAL4 activator (GAL4-AH) to nucleosomal DNA containing GAL4 binding sites, it acted in concert with the activator to destabilize the nucleosome. In the presence of GAL4-AH, transfer of H2A/H2B dimers occurred readily onto the carrier. GAL4 binding to the resulting H3/H4 tetramer was facilitated but this ternary complex dissociated upon addition of competitor DNA, the histones eventually being entirely displaced by the GAL4 activator. The disassembly of a nucleosome thus required a transcription factor with the potential to interact with nucleosomal DNA, a histone carrier with some specificity for H2A/2B dimers (RNA and polyglutamic acid could not substitute for nucleoplasmin in this assay) and finally, an unspecific competitor to accept the H3/H4 tetramers[39]. It is tempting to speculate that nucleosome disassembly may in part be a direct reversal of the assembly pathway, triggered by interaction of a transcription factor but making use of assembly factors. Whether nucleosome destabilization *in vivo* also involves histone acceptor molecules is difficult to test. Nucleosome disruption can be quickly reversed in highly regulated systems[42] and hence the histones are presumably stored close to the DNA, if not on the DNA in a subnucleosomal or highly modified particle of increased DNase I sensitivity.

Chromatin remodeling by GAGA factor requires ATP hydrolysis

The hypothesis that the GAGA factor, a transcription factor that recognizes GA-rich sequences within a large number of *Drosophila* promoters, is involved in organizing accessible chromatin structures was derived from detailed structural and functional analyses of heat shock promoters *in vivo*, carried out over the years by S. Elgin, J. Lis and coworkers[2,43]. Most recently, *in vitro* reconstitution experiments have lent support to this idea (see below). The GAGA factor was originally purified from *Drosophila* embryos as a transcription factor that interacted with multiple binding sites in a variety of promoters, including those of heat shock genes, developmentally regulated as well as housekeeping genes (ref. 44 and references therein). Its recent cloning[44] demonstrated that the single copy gene gives rise to a family of proteins, presumably by differential splicing of mRNA and post-translational modifications of the proteins. The results of the first functional analyses of the smallest protein (the 67 kDa species) originating from the GAGA gene, summarized below, has to be seen against the background of a whole range of related proteins that may or may not share certain features. Overexpression of GAGA^{67K} in tissue culture cells results in a substantial stimulation of promoters with multiple binding sites[44]. The increase of transcription in cell-free systems has been characterized as anti-repression rather than true activation, because GAGA factor is able to counteract the transcriptional inhibition brought about by abundant unspecific DNA-binding proteins in crude transcription extracts[45]. Binding sites for GAGA factor are central to the 'preset' structure of the hsp26 promoter, which includes accessible heat shock elements (HSEs), GAGA boxes, the TATA box and transcriptional start site. Accessible proximal and distal transcription factor binding sites are punctuated by a constitutively positioned nucleosome, thought to organize the promoter in space and thereby facilitating the interaction of distal Heat Shock Factor (HSF) with factors close to the start site (reviewed in refs 2 and 43). The 'preset' structure represents a state of alarm, characterized by the constitutive binding of TFIID and initiation of transcription by a polymerase molecule poised to continue transcription immediately upon activation by HSF[2,43]. Upon deletion of GAGA elements, the 'preset' structure is not established and the heat inducibility of the promoter is severely impaired, despite the presence of all HSEs. GAGA boxes are also spread over the first 200 base pairs upstream of the hsp70 start site, again demarcating the region found to be accessible to DNase I in nuclei[46]. While the detailed description of the protein-DNA interactions *in vivo* at wild-type and mutant promoters fitted with the hypothesis that the GAGA factor is a key determinant of the preset promoter[2], direct evidence for this hypothesis has come from very recent *in vitro* reconstitution experiments. Making use of a powerful chromatin assembly system derived from early *Drosophila* embryos[47] and recombinant GAGA^{67K}[44], Wu and collaborators analysed the interaction of GAGA^{67K} protein with the hsp70 promoter in chromatin[48]. Addition of GAGA factor to a promoter that had been assembled into nucleosomes with regular, physiological spacing resulted in the apparent disruption of the nucleosomes at and around the GAGA boxes, such that the region upstream of the transcriptional start site displayed hypersensitivity towards DNase I in a similar way to the natural promoter in nuclei. The interaction of GAGA factor also led to a significant repositioning of the flanking nucleosomes, consistent with the idea that the newly created hypersensitive site acted as a boundary for the re-alignment of neighboring nucleosomes. Remarkably, chromatin remodeling required ATP hydrolysis. When the target chromatin was purified by gel filtration chromatography, GAGA^{67K} worked inefficiently unless fresh hydrolyzable ATP was added[48]. The reaction was most efficient in the presence of multiple GAGA boxes spread over a distance of 150 bp, and suffered from progressive deletion of binding sites. This situation is reminiscent of the above-mentioned model reactions, which demonstrated that binding of GAL4-AH to multiple sites in a nucleosome can initiate at the loosely associated DNA at nucleosome edges and proceed cooperatively until even sequences close to the dyad axis of the particle are invaded[34]. Spread-out GAGA boxes over a considerable area ensure that there will always be a binding site in the linker, even when nucleosomes are assembled with no preferred position. A related study on the hsp26 promoter confirms and extends these results. Interaction of GAGA^{67K} with its binding sites in chromatin reconstituted in the *Drosophila* embryo extract resulted in a structure closely resembling the 'preset' promoter found *in vivo*: while the proximal and distal regulatory elements were kept accessible to factors, a loosely positioned nucleosome was localized between those elements at a position very similar to the one mapped in nuclei (G. Wall, P. Varga-Weisz, R. Sandaltzopoulos, and P. B. Becker, unpublished data). Again, setting the promoter was more efficient if hydrolyzable ATP was available. The purity state of the GAGA preparation used and the complexity even of purified chromatin, preclude statements about the possible involvement of cofactors in these reactions. It is unprecedented but revealing that the remodeling of preformed nucleosomes by GAGA^{67K} requires ATP hydrolysis, because a nucleosome as such is considered a fairly stable structure which, if associated with a strong positioning sequence, resists elevated salt concentrations and temperatures[49]. Immunofluorescence studies on polytene chromosomes using a polyclonal antiserum against GAGA^{67K} show that GAGA factor binds to many sites throughout the genome, suggesting a general role in chromatin organization[48]. In yeast, HSF binds constitutively to heat-inducible promoters and one therefore might predict that a presetting protein like the GAGA factor is superfluous. Indeed yeast HSF itself appears to be responsible for displacing nucleosomes, thereby creating accessible promoters[50].

The GAGA gene has also been cloned in a hunt for the Adf2 gene, a transcriptional regulator of the alcohol dehydrogenase promoter, and there is evidence that Adf2 and GAGA are identical (C. Benyajati, personal communication). The implication of this finding is that GAGA factors may also be able to interact with sequences other than GAGA boxes, such as the Adf2 binding site[51], and it will be interesting to learn how such diverse binding specificities are

achieved. The N-terminal 120 amino acids of GAGA factors show intriguing homology to the *Drosophila* transcription factors, tramtrack and broad-complex proteins[44]. Interestingly, this sequence similarity is also shared by a characterized enhancer of position-effect variegation, E(var)3-93D, which also binds to many sites on polytene chromosomes and may similarly be involved in establishing and maintaining open chromatin configurations[52]. Hsp70 and hsp26 promoters have both been classified as 'preset' promoters because their regulatory sequences are constitutively accessible and the action of the inducing factor does not involve an *ad hoc* nucleosome destabilization. The implication is that factors that organize preset promoters must not be able to actively disrupt nucleosomes, but could establish an accessible site by pre-emptive competition with nucleosome formation at the replication fork, when chromatin is transiently disrupted. The finding that GAGA factor, a strong candidate for presetting the hsp26 promoter, can remodel chromatin by dynamic competition means that the difference between 'preset' promoters and those requiring 'remodeling' may not be the mechanism of chromatin organization, but rather the availability of the active protein determinant. GAGA factors are present from the earliest stages of development[44].

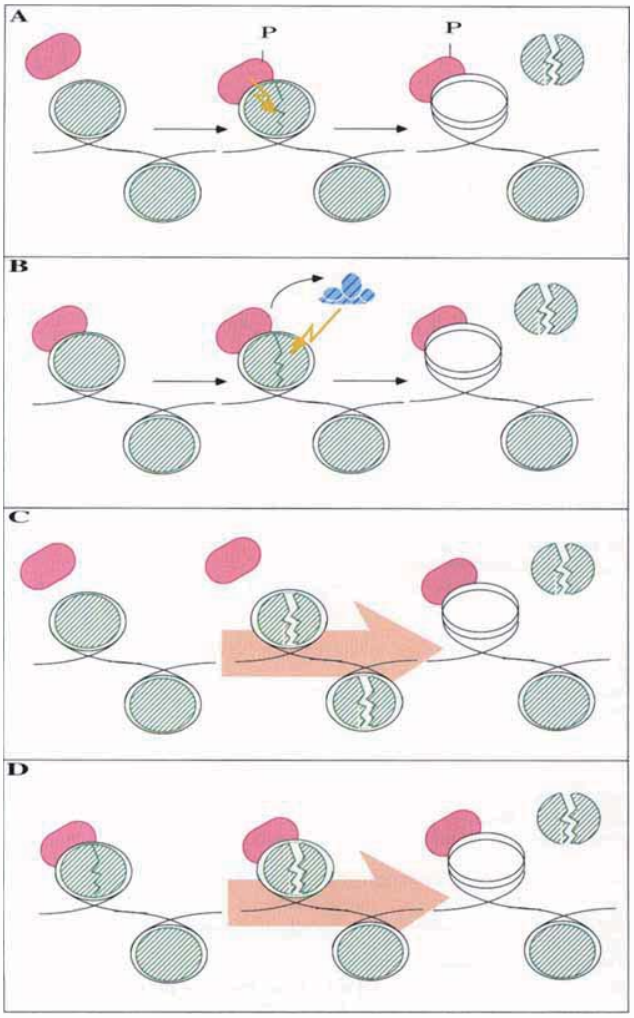

Fig. 1. Scenarios for nucleosome remodeling involving a 'Specificity Factor' and a 'Remodeling Factor'. Shaded particles (pink): Sequence-specific DNA binding protein. Hatched spheres (green): nucleosomes. For a description, see text.

Glucocorticoid receptor-triggered nucleosome disruption: something missing?

Activated glucocorticoid receptor (GR) is a paradigm factor involved in replication-independent remodeling of chromatin structure. Classical examples are the activation of the tyrosine aminotransferase enhancer (ref. 42 and references therein) and the opening of the MMTV promoter (reviewed in ref. 53). In the MMTV promoter, the well-positioned nucleosome B restricts the access of NF1/CTF and TFIID until hormone-loaded GR triggers an apparent disruption of the repressive nucleosome, enabling the crucial factors to activate transcription[53]. Nucleosome remodeling is accompanied by loss of histone H1 from the site of action[54], but the fate of the core histones is not known. The exact location of glucocorticoid response elements on the surface of the nucleosome modulates the affinity of GR to its binding sites[55]. Therefore, the precise positioning of nucleosome B is presumably the prerequisite for both the interaction of GR with the particle and their subsequent disruption *in vivo*. The preferred positions of nucleosome B can be reconstituted by salt gradient dialysis on a short piece of MMTV promoter DNA. Wrange, Hager, Beato and coworkers have shown that GR can bind to key response elements on the surface of the nucleosome resulting in a stable ternary complex (ref. 53 and references therein), but unfortunately GR will not remodel, disrupt or destabilize the nucleosome upon interaction. Clearly a crucial component is missing in these experiments, which by analogy with the experiments on GAL4, may turn out to be a histone carrier. Alternatively, a more physiological nucleosome reconstitution system of high complexity, such as the one from fly embryos[47], may provide as yet unknown auxiliary activities. When assayed in yeast, transcriptional activation by GR depended on functional SWI proteins, global activators of promoters that require dynamic remodeling of chromatin structure for transcription[56,11]. Related proteins exist in *Drosophila*[57] and mammals[58], where they also potentiate the action of GR. The SWI1, SNF2/SWI2, SWI3, SNF5 and SNF6 proteins act in concert as part of a large multisubunit complex in yeast[59,60] to prevent histone-mediated repression and chromatin changes at a responsive promoter depend on intact SNF2/SWI2 and SNF5 proteins[61]. The finding of extensive sequence similarity between the SNF2/SWI2 protein and known DNA or RNA helicases has provoked models suggesting that topological changes brought about by helicases might be triggers for chromatin reorganization[11], but the functional significance of this sequence similarity has not been demonstrated. Remarkably, the above-mentioned *Drosophila maleless* protein, associated with the transcriptionally hyperactive male X chromosome, also contains convincing similarity to DNA-dependent ATPases and helicases (ref. 21 and references therein).

Site-specific nucleosome remodeling

Naive models that try to explain how nucleosome remodeling may be achieved can be grouped into two classes, depending on whether a single transacting factor is sufficient for remodeling or whether two or more factors need to cooperate. In the cases discussed above, a single sequence-specific nucleosome binding protein was apparently not sufficient to modify nucleosome structure. GAL4-mediated nucleosome disassembly depended on histone carriers. Remodeling by GAGA factor required ATP hydrolysis and this requirement has not yet been traced to the factor itself. GR obviously needs assistance by other factors, which may turn out to be SWI-like proteins.

The selectivity of nucleosome targeting is most probably determined by the exact position of the nucleosome with respect to the binding site of a transcription factor, which may be close to or even on the nucleosome. In the latter case the protein must be able to bind to nucleosomal DNA, but fruitful interaction may require a precise rotational and/or translational positioning of the nucleosome and a permissive histone modification pattern. With GAL4, GAGA factor, GR and like proteins in place as 'specificity factors', a cooperating 'remodeling factor' would not need to display similar selectivity. Splitting the requirements for nucleosome remodeling into two separate components would expand the opportunities for regulation and create combinatorial flexibility. Fig. 1 shows cartoons of remodeling scenarios of the kind that, seeking inspiration, one may scribble onto a sheet of paper along with many others. They all involve accessory 'remodeling factors' with different degrees of specificity in addition to the 'specificity factor'. In (A), phosphorylation of the specificity factor modulates its ability to interact with a nucleosome in a way that leads to its destabilization. In (B), the binding of the specificity factor to nucleosomal DNA does not lead to its disruption, but it guides an unspecific remodeling unit to the target site. This remodeling factor, unable to bind DNA by itself, would not be able to find its target unless directed by the DNA-bound specificity factor. A general remodeling factor of this type might be able to interact with many different specificity factors. The scenarios in (C) and (D) assume that chromatin in a physiological context is much more dynamic than is generally appreciated. Waves of transient nucleosome unfolding or partial disassembly, a direct reversal of the assembly reaction with which it may be in a dynamic equilibrium, may create windows of opportunity, of increased chances for competitive DNA binding by transcription factors in the absence of replication. A *trans*-acting factor may take advantage of such a local 'breathing' of nucleosomal structures, trap the partially disrupted complex or shift the equilibrium towards disassembly (C). Alternatively, the interaction of a specificity factor with a nucleosome might induce a conformational change in the particle that, while not sufficient *per se* for disruption, would render the nucleosome fragile and prone to break when the wave comes (D). There is only a little experimental evidence to support such a view. However, even well-positioned nucleosomes appear to have more dynamic properties than previously thought (ref. 62 and references therein). Intriguing findings that relate chromatin remodeling to the action of DNA helicases, which might influence chromatin topology locally and over a considerable distance, indicate that after having accumulated solid knowledge about the static aspects of chromatin structure, we will need to adopt a flexible attitude towards chromatin dynamics in order fully to understand how transcription is regulated in and by chromatin.

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