Minireview

Nucleosome Transactions on the Promoters of the Yeast GAL and PHO Genes*

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The GAL and PHO genes of yeast provided some of the earliest evidence for specific nucleosome changes on eukaryotic promoter regions, and they continue to contribute unique insights to this emerging area. These nutrient-regulated systems possess major advantages for chromatin studies. Gene activity is tightly regulated and easily manipulated; firm genetic foundations provide strong functional correspondence for biochemical analyses. The promoter region nucleosome changes (“transactions”) to be discussed here include disruption, which refers to the loss of nucleosome structure observed when transcription is activated, and reorganization, which refers to the regeneration of promoter region nucleosome structure and is associated with gene inactivation. Results are from in vivo or nuclear chromatin analyses unless otherwise stated. Other recent reviews also cover some of these and related subjects (1-7).

Basic Relationships between Gene Expression and Promoter Region Nucleosome Changes on PHO and GAL

PHO

The incisive analysis of PHO5, mainly carried out by Hörz and co-workers (1, 3), has made the PHO system a major chromatin model. PHO5 encodes an acid phosphatase. It is regulated by extracellular [phosphate], repressed by high phosphate-induced to expression (“derepressed”) by phosphate deprivation (Table I). Induction depends on the major, specific phosphate/induced to expression (“derepressed”) by phosphate deprivation by extracellular [phosphate], repressed by high phosphate/induced to expression (“derepressed”) by phosphate deprivation (Table I). Induction depends on the major, specific inducer. Pho2p (1, 3). Pho4p binds to the activation sequence; gly/lac, glycerol/lactate; PIC, preinitiation complex; TBP, TATA binding protein; bp, base pair(s).

Upstream Nucleosomes Function in PHO5 Regulation and Control UASp2 Accessibility—1 The upstream nucleosomes help repress PHO5 because their depletion, achieved by altering histone stoichiometry, allows significant PHO5 expression under repressed conditions (15). Moreover, the transition to the disrupted (activated) state apparently depends on upstream nucleosome stability because replacement of nucleosome 2 DNA with a sequence that can form a hyperstable nucleosome results in PHO5 inhibition and persistence of the inactive nucleosome array structure under induced conditions (16). 2 Overexpression of truncated Pho4p derivatives can force Pho4p binding to a UAS located in the non-nucleosomal (UAS)1 site but not to a UAS located in nucleosome 2 (11, 14). Thus, nucleosome 2 very strongly restricts UAS2 access.

Nucleosome Disruption Is Probably Initiated by Changes in Pho4p Location and Involves Pho2p/UASp2—Pho4p is predominantly cytoplasmic in repressed cells but predominantly nuclear under induced conditions (9). Higher nuclear [Pho4p] and an enhanced Pho4p-UAS affinity in derepressed cells (11) may be sufficient to trigger disruption. Pho4p probably initiates this process by binding to UAS1 (11), possibly in concert, and cooperatively, with Pho2p binding to its strong site that overlaps UAS1 in the nucleosome-free region (Fig. 1A). This binding could serve to anchor Pho4p while its activation domain mediates nucleosome disruption and put Pho2p in a position to aid in the process. For example, Pho2p may help expose the Pho4p activation domain by freeing it from an intramolecular interaction with the Pho4p DNA binding domain (17). However, neither Pho2p nor UAS1 is absolutely required for disruption because in pho2 (13) or UAS1 (11) strains, overexpressed Pho4p can itself produce disruption. Disruption in the UAS1 strain does require a functional UAS2 (11). This result and the observation that Pho4p-UAS2 binding is only observed in disrupted chromatin (11) suggests that Pho4p-UAS2 binding and nucleosome disruption are tightly linked.
**PHO5** disruption can be viewed as a multicomponent reaction in which the Pho4p activation domain, Pho4p-UAS binding, Pho2p-DNA binding, and other contributions (see below) cooperate to provide enough energy to disrupt the upstream nucleosomes. This reaction must be only moderately favorable because the presence of a hyperstable nucleosome in the array can prevent disruption (16). Mass action effects on this reaction might explain why overexpressed Pho4p can itself produce disruption (Pho2p-UASP1 strains). The four-nucleosome array disrupts as a unit (11); these nucleosomes may be structurally linked and their disruption cooperative. Chromosomal context does not play a major role because the array and its disruption occur as usual when PHO5 is in a CEN plasmid (12). We can look forward to further analysis of this intriguing chromatin transition.

**GAL**

The **GAL** genes encode the enzymes and regulators needed to utilize galactose as a carbon source (2, 18). The structural genes (**GAL1–10**, −7) are strongly induced by galactose through the specific activator Gal4p (Table I). Gal4p activates transcription through a domain in its C terminus while bound, through the specific activator Gal4p (Table I). Gal4p activates disruption occur as usual when **PHO5** is in a CEN plasmid (12). We can look forward to further analysis of this intriguing chromatin transition.

**The UASG Are Constitutively Accessible to Gal4p—**The upstream chromatin regions on **GAL** genes (**GAL1–10**, −7, −80) contain a sizeable (−170 bp) stretch of DNA that is permanently nucleosome-free (19–22), in every carbon source, plus or minus Gal4p/Gal80p (23). The UASG on **GAL1–10** (Fig. 1B), **GAL7** (21), and **GAL80** (22) all lie completely within the non-nucleosomal regions. Thus, Gal4p can bind to all of these UASG without disrupting nucleosomes. The ability of Gal4p to access and bind to the UASG in gly/lac helps poise cells for rapid inducibility, thus enabling a quick switch to the better carbon source galactose (2). On **PHO5**, conditional UASp2 accessibility helps implement repression (see above). Restricted UAS accessibility and Pho4p subcellular location probably control Pho4p-UASp binding; Pho4p levels are the same under activating or repressing conditions (24). Gal4p-UASG binding appears to be determined mainly by Gal4p levels (2).

**Galactose Induction Causes Gal4p-dependent Disruption of Upstream Nucleosomes on **GAL1–10**, −7, and −80—**In the inactive state (poised or repressed), positioned nucleosomes cover the GAL10, −7, and −80 TATA and the **GAL1** and −80 transcription start sites and surround the **GAL1** TATA (19–22) (Fig. 1B). These nucleosomes help repress gene activity because nucleosome depletion in non-galactose carbon sources allows some TATA-dependent **GAL1** expression (25). Galactose induction triggers the Gal4p-dependent disruption of all of these upstream nucleosomes (19–22, 26–28). For **GAL1–10** nucleosome B, it is known that disruption depends on the transcription activation domain of Gal4p (27); this is likely to be true for the other upstream nucleosomes, for example A and C (Fig. 1B), which are disrupted simultaneously with B (28). Nucleosome disruption exposes the TATA to various exogenous probes (19–22, 26–28) and thus should also enhance its exposure to TBP/PIC, thereby facilitating transcription initiation. Also, the first DNA melting for **GAL10** and **GAL1** transcription occurs within disrupted nucleosome A and C regions (29). Release of the negative supercoiling restrained by those nucleosomes might aid this initial strand separation. Note that the DNA binding and nucleosome disruption functions of Gal4p act at sites that are distinct and almost certainly quite spatially distant in the chromatin structure. In Pho4p, these functions act, at least in part, on the same chromosomal region (UASp2).

In Gal4p, DNA binding and disruption are independent, i.e., one can occur without the other (gly/lac); in Pho4p these functions seem to be linked.

**Upstream Nucleosomes Are Readily Reorganized; the Process Is Gal80-dependent**—During the initial steps of nuclear isolation (cell harvest/spheroplast preparation) in our well defined wild type strain, the disrupted structure of the induced **GAL1–10** and −80 upstream regions is completely reorganized. However, in isogenic gal80Δ mutants under the same conditions, this upstream nucleosome reorganization does not occur (28). Thus, Gal80p must be required for the reorganization observed in wild type. This reorganization produces the typical inactive (present in non-galactose carbon sources) upstream nucleosome structure and probably involves the same process that normally reorganizes these regions in response to galactose absence (28).

**Reorganized Upstream Nucleosomes Can Be Redizontally Disrupted; Disruption/Reorganization Is Rapid and Limited to Upstream Nucleosomes**—The **GAL1–10** and −80 upstream nucleosomes that are reorganized during spheroplast preparation can be disrupted by simply incubating the prepared spheroplasts in galactose. This disruption resembles in vivo disruption in several ways (28) and is probably carried out by the same process. To observe disruption, spheroplasts must be prepared from induced cells. This probably indicates that other steps, such as recruitment of transcription factors/disruption machinery, are required to set up the readily disrupted state.

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**Table I. Regulators and gene activity**

| Gene   | Major promoter | Major activator | Major repressor | Induction conditions | Inactive in |
|--------|----------------|-----------------|-----------------|---------------------|-------------|
| **PHO5** | UASG 1 | Pho4p | Pho80p | Low Pi | High Pi |
| **GAL1–10** | 4 UASG | Gal4p | Gal80p | Galactose | Glu (repressed) 
  gly/lac (poised) |

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**FIG. 1. Organization of the PHO5 and GAL1–10 upstream regions.** Along the lines are located the UAS, TATA (boxed T), and transcription start sites (thin arrows). Major tick marks on the lines lie at 100-bp intervals. Below the lines, nucleosome positions are located to scale by rectangles. Stippled rectangles are the nucleosomes that are disrupted by gene activation; open rectangles are other positioned nucleosomes. A, PHO5. The strong Pho2p binding sites are located by thick vertical arrows, and nucleosomes that are subject to disruption/reorganization are labeled −1 to −4. B, **GAL1–10**. Disrupted/reorganized nucleosomes are labeled A–C.
GAL1–10 and −80 upstream nucleosomes are completely disrupted within 10–15 min in spheroplast treatments and completely reorganized sometime within a 1–2-h protocol (28). PHO5 upstream nucleosome disruption is well under way within 15 min after shifting pho80Δ cells from 24 °C (permissive for Pho80p function/PHO5 repressed) to 37 °C (restrictive for Pho80p function/PHO5 induced). The reverse temperature shift in 80ts mutants or phosphate incubation of spheroplasts from induced wild type cells triggers reorganization to the inactive array structure within 15 min (30). Thus, nucleosome transactions of both types can occur rapidly, without the need for DNA replication or cell growth (30).

The disruption/reorganization behavior described above is apparently restricted to upstream nucleosomes because the induced pattern on the GAL1 coding region (21, 31) is not affected at all during these procedures that so radically alter the upstream regions (28). This restriction is consistent with the likely localization of the transaction-mediating factors Gal4p/Gal80p to the upstream regions. Gal4p/Gal80p probably carry out these nucleosome transactions via the constitutive, stoichiometric complex they are thought to form in vivo (2).

Some More General Considerations

The Role of Regulatory Factors in Nucleosome Transactions—The specific GAL and PHO regulators may promote transactions indirectly, by acting through other factors. For example, Pho4p may simply recruit RNA polymerase II holoenzyme to carry out the PHO5 nucleosome disruption (32). One of the several multiprotein complexes known to destabilize nucleosomes (4, 5, 7), e.g. RSC (33), may also help in disruption. Specific regulators might also directly participate in transactions, acting through their activation domains or other regions. For example, genetic analysis of the Gal4p C-terminal (activation) domain has defined a specific activation “face” that contains Thr and Tyr (two each) as the major residues (34). If these residues were to target the hydrogen-bonding interactions that stabilize the octamer, between H2A-H2B dimers and the H3-H4 tetramer (35), Gal4p could cooperate directly with other factors in disrupting nucleosomes. It is also important to consider that specific regulators probably carry out nucleosome transactions and gene activation while in some kind of organized three-dimensional superstructure (36), which will influence their operation. For example, very little Gal4p can be isolated from cells, presumably because it is present in an insoluble structure in vivo (2).

On both PHO and GAL, the upstream nucleosome transactions alter TATA exposure and thus can affect the ability of TBP/PIC to access the TATA, a process crucial to transcription initiation. These nucleosome transactions might therefore be closely regulated; by regulating both (disruption/reorganization) the cell can use nucleosome occupation of the TATA as a controllable switch, perhaps a fairly late one, in the activation pathway. Disruption permits activation. Reorganization may be part of the mechanism that deactivates expression in response to the appropriate inactivating signal(s). This switch could be controlled by regulator-sensitive competition between nucleosomes and the TBP/PIC; activators facilitate TBP/PIC occupation (and nucleosome removal); negative factors like Gal80p promote nucleosome occupation. For example, the ability of Pho4p and Gal4p to activate transcription correlates directly with their ability to disrupt upstream nucleosomes (14, 27). Also, TBP mutants that bind less well to DNA, and thus could compete less well with nucleosomes, decrease the ability of Gal4p to activate transcription (37). Some of these features might account for the uniqueness of upstream transactions (compared with those on coding regions, see above).

The Role of Histones in Nucleosome Transactions—Histones play specific roles in GAL and PHO, expression through their N-terminal tails. For example, removal of H4 tails decreases the level of induced GAL1 and PHO5 expression −20- and −4-fold, respectively (38). Removal of H3 tails has little effect on PHO5 but causes GAL1 to be hyperexpressed under induced conditions (39). These tails are not involved in the histone-histone interactions that hold together the octamer (35) and thus are free to engage in interactions with intranucleosomal DNA, linker DNA, other nucleosomes, or non-histone proteins.

Acetylation of the lysines in H3 and H4 tails has long been linked to transcriptionally active chromatin. Acetylation could destabilize nucleosomes and thus facilitate disruption, because less positively charged, acetylated histone tails should interact more weakly with intranucleosomal DNA. However, removal of tails, e.g. of H4, should also diminish these interactions and thus facilitate disruption and therefore gene activation. Instead, PHO5 and GAL1 transcription decreases (38). The inhibitory effects of H4 tail loss might reflect the specific involvement of these tails in the nucleosome disruption that accompanies gene activation. For instance, they could be contacts for nucleosome-disrupting machinery. H4 tail loss results in increased protection at the TATA-proximal end of GAL1–10 nucleosome B, suggesting that these tails normally prevent the formation of a repressive (nucleosome) structure and thus maintain transcription factor access around the TATA (40). These mechanisms might require acetylated H4 tails.

The hyperexpression (GAL1) produced by H3 tail removal suggests a different function for these tails. The level of hyperexpression is roughly the same as the hyperexpression caused by Gal80p loss, −2–3-fold (2). H3 tails could thus play a role in Gal80p-dependent nucleosome reorganization; their acetylation might inhibit reorganization and thus favor the disrupted state. Gal80p modulates the level of induced GAL1 expression (2), and this is presumably why GAL80 is more highly expressed (5–10-fold) in galactose, even though Gal80p inhibition of Gal4p is relaxed and GAL genes are activated. This modulation of GAL1 expression might be implemented by enhancement of the potential for Gal80p-dependent reorganization of upstream nucleosomes through increased Gal80p levels.

What Is “Disruption”?—Does disruption reflect complete octamer loss, partial histone loss (most likely H2A-H2B (41)), or some kind of conformational change? Complete loss of H2A-H2B, leaving only the H3-H4 tetramer, should expose DNA near nucleosome ends and for −20 bp around the dyad (42). This kind of change is observed for the disrupted, TATA-containing nucleosome on the modestly induced GAL80 gene (28).

However, on GAL1–10 or PHO5, disruption results in strong cleavage throughout nucleosomal regions A–C, or −1 to −4, and the chromatin digest pattern resembles a naked DNA pattern. This suggests octamer loss. On the weakly regulated PHO5, derepression causes only instability (unfolding?), and partial accessibility increases in the upstream nucleosomes (43). Upstream nucleosome disruption may thus involve different types of changes on different genes, depending perhaps on expression level or tightness of regulation. This variation might reflect the sequential nature of disruption, i.e. loss of H2A-H2B dimers first and then the H3-H4 tetramer, and/or the presence of multiple disruption pathways (4, 5, 7).

A conformational change that exposes DNA to cleavage without core histone loss has been suggested for the disruption of a nucleosome on the mouse mammary tumor virus promoter (1, 7). Other changes that could expose nucleosomal DNA without histone loss include a partial peeling away of DNA from the octamer, as suggested to occur during RNA polymerase transcription through a nucleosome (44) and recently proposed as a model for factor binding to nucleosomal DNA (45) and nucleo-
some sliding along DNA (46). Sliding may occur on the GAL1 coding region during induction (21) but probably does not explain the GAL1–10 upstream region changes (28).

Nucleosomes possess inherent instability. For example, in vitro at physiological salt concentrations, there is spontaneous, low level octamer loss from purified polynucleosomal templates (47). In vivo disruption mechanisms may depend on, and amplify, these inherent tendencies. Examples of such inherent pathways may be 1) the peeling off of nucleosomal DNA triggered in vitro by DNA binding proteins (45) and 2) sequential histone loss (H2A-H2B and then H3-H4), which is the exact reversal of the nucleosome assembly pathway (5, 35, 47, 48).

Nucleosome Transitions May Require Numerous Cooperating Processes—Nucleosome transitions probably involve bidirectional reactions whose “equilibrium” position can be shifted, in either direction, by factors or processes that affect the participating species. For example, in disruptions that involve histone loss, the presence of an acceptor for the dissociated histones should thermodynamically favor H2A-H2B and octamer loss from DNA. The rapidity and reversibility of PHO and GAL transitions suggest that dissociated histones could remain nearby, perhaps bound to histone acceptors. Potential histone acceptors include: nucleosome-disrupting complexes (4, 5, 7, 49), which might provide at least transient histone binding sites; such as UASp2 for Pho4p or the TATA for transcriptional control, regulating UAS and TATA accessibility. Transactions may utilize subsidiary cellular factors, as well as specific features (N-terminal tails) of the histones themselves. It will be important to determine precisely what kind of change(s) occurs in nucleosome disruption. This will provide insight on possible mechanisms and what kind of subsidiary factors or processes are needed to support the transactions.

location of nucleosome disruption functions within activation domains will help distinguish the multiple roles of activators and how these roles are implemented.

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