Chromatin Higher Order Folding: Wrapping up Transcription

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Eukaryotic genomes are organized into condensed, heterogeneous chromatin fibers throughout much of the cell cycle. Here we describe recent studies indicating that even transcriptionally active loci may be encompassed within 80- to 100-nanometer-thick chromonema fibers. These studies suggest that chromatin higher order folding may be a key feature of eukaryotic transcriptional control. We also discuss evidence suggesting that adenosine-5′-triphosphate–dependent chromatin-remodeling enzymes and histone-modifying enzymes may regulate transcription by controlling the extent and dynamics of chromatin higher order folding.

Compaction of eukaryotic genomes into condensed chromatin fibers is required to fit over a meter of DNA within the limited volume of the nucleus; consequently, this compacted structure is inherently repressive to processes that require access to the DNA sequence. The role of higher order chromatin folding in transcriptional control received the lion's share of interest in the early 1980s [for examples see (*1*, *2*)], but only recently has this key issue been seriously revisited. Recent advances in our ability to assemble model chromatin in vitro and to identify posttranslational chromatin modifications as key components of gene expression have enhanced interest in the interplay between chromatin structure and transcription. Although substantial strides have been made toward an understanding of basic chromatin structure, much of the detail surrounding "higher order" structure— chromatin structure beyond the canonical "30-nm" fiber familiar from textbooks— remains partially or completely uncharacterized.

In this review, we discuss the basic components of chromatin, their role in the structure of the 30-nm chromatin fiber, the limited details known about chromatin structure beyond this basic fiber, and, finally, how the regulation of these structures might serve as a key element of transcriptional control.

Chromatin Basics

The basic building block of chromatin is the nucleosome, which contains 147 base pairs (bp) of DNA wrapped in a left-handed superhelix 1.7 times around a core histone octamer [two copies each of histones H2A, H2B, H3, and H4 (*3*)]. Each core histone contains two separate functional domains: a signature "histone-fold" motif sufficient for both histone-histone and histone-DNA contacts within the nucleosome, and NH_2-terminal and COOH-terminal "tail" domains that contain sites for posttranslational modifications (such as acetylation, methylation, phosphorylation, and ubiquitination). Although these histone tails are mostly unresolved in the crystal structure of the nucleosome (*3*), they appear to emanate radially from the nucleosome, conveniently positioned to associate with "linker" DNA residing between nucleosomes or with adjacent nucleosomes (*4*). In addition to the core histones, metazoan chromatin also contains linker histones (such as histone H1), which bind to nucleosomes and protect an additional ~20 bp of DNA from nuclease digestion at the core particle boundary. Linker histones are not related in sequence to the core histones, but they also contain a globular domain flanked by NH_2-terminal and COOH-terminal tail domains (*5*). Although only the linker histone globular domain is essential for binding to nucleosomes, the tail domains are believed to be important for linker histone roles in chromatin folding (*6*).

Folding Properties of a Basic Fiber

In the genome, thousands of nucleosomes are organized on a continuous DNA helix in linear strings separated by 10 to 60 bp of linker DNA (Fig. 1). Thus, the lowest functional unit of chromatin might actually be considered the "nucleosomal array." Much of our understanding of the solution state behavior of nucleosomal arrays has come from biophysical analyses of model arrays reconstituted with purified histones and a DNA template composed of 12 tandem repeats of a 208-bp nucleosome-positioning sequence (the "208-12" array) (*4*). In low-ionic-strength buffer, these model arrays sediment in the analytical ultracentrifuge in a fairly uniform distribution that can be modeled as a fully extended "beads-on-a-string" fiber. In contrast, the addition of 1 to 2 mM divalent cation establishes a heterogeneous population of folded arrays, where the most condensed species can be modeled as a compact 30-nm fiber. At higher cation concentration, the arrays also self-associate to form high molecular weight oligomers that may mimic the fiber-fiber interactions that drive formation of thick (>30 nm) chromatin fibers in vivo. Consistent with the idea that the histone tails mediate key nucleosome-nucleosome interactions that are essential for chromatin structure in vivo (*3*, *4*), the histone tails are critical for both the intramolecular folding of arrays and for fiber-fiber interactions observed in vitro.

Although condensation is an intrinsic property of the nucleosomal array, the binding of linker histones stabilizes both intramolecular folding and fiber-fiber interactions. For instance, the addition of linker histone H5 (a histone H1 variant) to nucleosomal arrays produces a homogeneous, fully compacted array rather than the heterogeneous collection of conformations observed in the absence of H5 (*7*). The removal of the histone tails still blocks condensation even in the presence of linker histone, implying that linker histones stabilize an intrinsic tail-mediated condensation pathway rather than stimulating condensation through a parallel pathway (*8*). These properties of linker histones suggest that they may lock down regions of chromatin into a condensed state that serves as a foundation for even higher order structures.

Despite over 20 years of effort, the structure of a condensed chromatin fiber remains a contentious issue. Over the years, several competing models have been proposed [for detailed treatments of the various models see (*6*, *9*, *10*)]. Each model requires distinct assumptions regarding the linker DNA conformation, the positioning of linker histone H1 within the fiber, and the mass per unit length of the fiber. To date, no single model fits the preponderance of the data, with contradictory evidence supporting one or another model. In fact, the disparity of the available data has caused some to question whether the condensed state contains any regular structure at all (*11*).

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Dynamics of the Basic Fiber: Roles for Chromatin-Remodeling Enzymes

Although the precise structure of a folded chromatin fiber is not known, an appreciation of the intrinsic folding properties of nucleosomal arrays is crucial, given that in vitro transcription studies on nucleosomal templates commonly employ Mg^{2+} concentrations (~6 mM) that are expected to drive substantial intramolecular folding and intermolecular self-association. Numerous studies have demonstrated that the positioning of nucleosomes over key promoter elements can repress transcription by RNA polymerase II in vitro (*12*, *13*), but the contribution of chromatin folding to transcriptional regulation has received little attention since the early 1980s. What is clear is that the functioning of gene-specific transcriptional activators often involves the targeting of two types of chromatin-remodeling enzymes to the promoter region, an adenosine-5′-triphosphate (ATP)–dependent, SWI/SNF-like complex and a histone acetyltransferase (HAT). These two types of enzymes appear to act synergistically to establish a local chromatin structure that is permissive for subsequent events (*14*). Recent studies have tended to illustrate how SWI/SNF-like enzymes move or alter individual nucleosomes (*15*) or how the action of HATs controls the binding of other nonhistone chromatin proteins [that is, the histone code hypothesis (*16*)]. Do chromatin-remodeling enzymes also facilitate transcription by contending with chromatin folding? Consistent with this possibility, moderate levels of histone acetylation do destabilize the folding of model 208-12 nucleosomal arrays, and this inability to fold directly correlates with enhanced transcriptional elongation by RNA polymerase III (*17*). Conversely, transcriptional repression by histone deacetylases may involve stabilization of chromatin higher order folding through deacetylation of the histone NH_2-terminal tails.

Although it is clear that the p300/CBP HAT plays additional roles in the transcription of nucleosomal templates (*18*), a recent study supports a role for it in contending with chromatin folding. Laybourn and colleagues assembled the viral HTLV-1 promoter region into a randomly positioned nucleosomal array with recombinant histones that contained or lacked their NH_2-terminal tails (*19*). Transcription from the intact nucleosomal array required the cyclic adenosine 3′,5′-monophosphate response element–binding factor (CREB) activator, the viral transcription factor Tax, p300/CBP, RNA polymerase II, and other basal transcription factors. Removal of the histone tails enhanced the activation of transcription by Tax and CREB and eliminated the requirement for p300/CBP. Thus, in this in vitro system, the HAT activity of p300/CBP is equivalent to histone tail removal. Because histone tail removal eliminates the folding of arrays but has little, if any, effect on nucleosome structure, these data suggest that nuclear HATs such as Gcn5p, PCAF, and p300/CBP can enhance transcription by disrupting the nucleosome-nucleosome interactions that stabilize chromatin folding.

Several studies also imply that ATP-dependent remodeling by SWI/SNF-like enzymes might involve disruption of chromatin folding. In yeast, the genes that encode many of the subunits of the ySWI/SNF complex were initially identified in genetic screens as positive regulators of transcription (*20*). In a recent study, it was discovered that ySWI/SNF plays a more global role in the transcriptional activation of genes expressed late in mitosis (*21*). These results led to the suggestion that ATP-dependent remodeling might lead to a localized disruption of mitotic condensation. A mitotic role for SWI/SNF-like complexes is not restricted to yeast, as *Caenorhabditis elegans* SWI/SNF is required in late mitosis for the subsequent asymmetric division of T cells during early development (*22*).

Additional biochemical evidence for ATP-dependent disruption of chromatin folding comes from recent analyses of nucleosomal arrays that harbor Sin^- versions of histone H4 (*23*). Sin^- histones were first identified as a group of yeast mutants affecting histone H3 or H4 that restored transcription in the absence of SWI/SNF. Similarities between the properties of SWI/SNF remodeling in vitro and the in vivo phenotypes of Sin^- mutants (*24*) led to the initial suggestion that Sin^- histones may lead to a nucleosome structure that mimics SWI/SNF-dependent nucleosome disruption. Analysis of model 208-12 arrays demonstrated that a Sin^- histone H4 eliminates Mg^{2+}-dependent intramolecular folding of nucleosomal arrays. Thus, these data suggest that Sin^- versions of histones may alleviate the need for SWI/SNF in vivo by disrupting higher order chromatin folding and, furthermore, that SWI/SNF may act primarily at the level of the folded chromatin fiber in vivo, rather than on single nucleosomes (*23*).

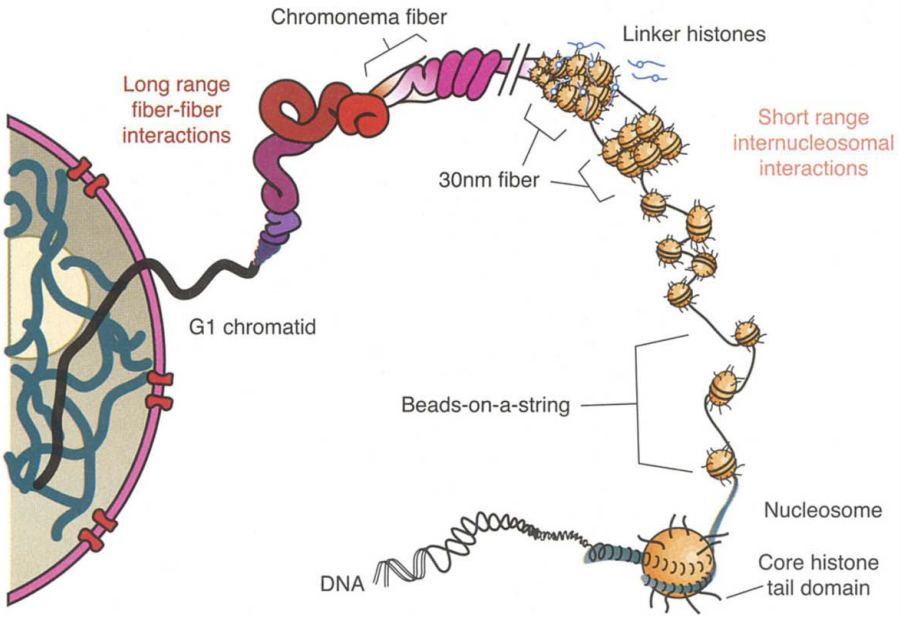

Fig. 1. Multiple levels of chromatin folding. DNA compaction within the interphase nucleus (depicted at left) occurs through a hierarchy of histone-dependent interactions that can be subdivided into primary, secondary, and tertiary levels of structure. Strings of nucleosomes compose the primary structural unit. Formation of 30-nm fibers through histone tail–mediated nucleosome-nucleosome interactions provides a secondary level of compaction, whereas tail-mediated association of individual fibers produces tertiary structures (such as chromonema fibers). This figure is adapted from figure 1 of (*4*).

Chromonema Fibers: Beyond the Coil

What is the structure of the chromatin fiber in vivo? Do 10-nm, bead-on-a-string chromatin fibers exist, or are they only present in vitro in low-ionic-strength environments? In vitro, the concentration of divalent ions required to induce 30-nm fiber formation is really quite modest (1 to 2 mM). In fact, the estimated interphase nuclear concentrations of Ca^{2+} and Mg^{2+} ions [4 to 6 mM and 2 to 4 mM, respectively (*25*)] are not only expected to condense chromatin fibers but also should drive substantial fiber-fiber interactions (*26*). For this reason, when considering how chromatin could affect gene expression, one must consider that chromatin primarily exists in a highly ordered state in vivo (*27*). Reinforcing this point are elegant light and electron microscopy studies of mitotic and interphase chromosomes. Fiber sizes ranging from 100 to 300 nm in diameter were observed throughout mitotic

chromosomes (*28*), and electron micrographs of interphase chromosomes also displayed a substantial amount of 100-nm-wide fibers (*29*). These very large fibers are unstable when nuclei are prepared in the absence of divalent cations or polyamines (*29*), mimicking the Mg^{2+} dependence of folding and self-association seen for nucleosomal arrays in vitro.

Belmont and Bruce carefully followed changes in these "chromonema" fibers during mitotic exit into interphase, where they observed regional decondensation initially through a 100 to 130-nm intermediate fiber and eventually into a 60 to 80-nm "chromonema" fiber that could be traced for 1 to 2 μm. Only occasional stretches of 30-nm fiber were seen (*30*). For some perspective on the size of these fibers, consider that a 30-nm fiber running across the 100-nm width of a chromonema fiber would package over 10,000 bp of DNA (*31*). Thus, a single chromonema fiber could conceivably contain the entire enhancer and promoter regions of even a mammalian gene.

Chromonema Fiber Dynamics: Transcription

These studies provide evidence of an extensive yet dynamic structural organization beyond the 30-nm fiber. But how do these structures influence gene function? Is transcription actually occurring on chromonema fibers, or are transcriptionally active regions less condensed? To answer these questions, Tumbar *et al.* used a mammalian cell line that contained a long, integrated array of LacI-binding sites (Fig. 2) (*32*). When imaged in living cells by decoration with a LacI–green fluorescent protein (GFP) fusion protein, this 90-Mbp tract appears as a single dot. In contrast, the foci decondensed when cells were transfected with an expression vector that produced a fusion of LacI-GFP to the VP16 transcriptional activation domain. The decondensed LacI tract appeared as an extended ribbon estimated to be 80 to 100 nm in diameter that coiled throughout a considerable volume of the nucleus. The 100-nm fiber colocalized with regions of bromodeoxyuridine–uradine 5′-phosphate incorporation, suggesting active transcription within the site, and recruitment of multiple HATs and increased histone acetylation were also observed. Thus, these data suggest that a 100-nm fiber may be the basic unit of higher order structure that is competent for gene expression (*32*). Because RNA polymerase II inhibitors did not block formation of the 100-nm fibers, the structural changes observed are not caused by transcriptional activity but more likely precede transcriptional activation at these sites.

One concern with these studies is that these tandem LacI arrays are artificial and, thus, their behavior might be aberrant. To look in a more natural context, Mueller *et al.* (*33*) examined the behavior of a tandem array of mouse mammary tumor virus (MMTV) promoters driving expression of a *ras* gene. Array structure and transcription were examined using a glucocorticoid receptor–GFP (GR-GFP) fusion protein to decorate the array in live cells and fluorescence in situ hybridization of fixed nuclei to verify transcription. The behavior of this MMTV array was dynamic. The addition of a GR agonist led to rapid (1 to 3 hours) decondensation of the arrays, with a rate that paralleled accumulation of *ras* mRNA. Even the decondensed, transcriptionally active arrays retained at least 50-fold more condensation than naked DNA, suggesting maintenance of at least a fully condensed 30-nm chromatin fiber. Therefore, both the LacI-GFP-VP16 activator and GR-GFP can drive local decondensation of a chromatin fiber. Because VP16 and GR are known to recruit either HATs or ATP-dependent chromatin-remodeling complexes, it is tempting to speculate that these activities play key roles in these decondensation events. In fact, the recruitment of transcriptional coactivators, including HATs and human SWI/SNF, was seen during MMTV array induction (*33*), and extensive histone acetylation accompanied VP16-dependent decondensation of the arrays examined by Tumbar *et al.* (*32*).

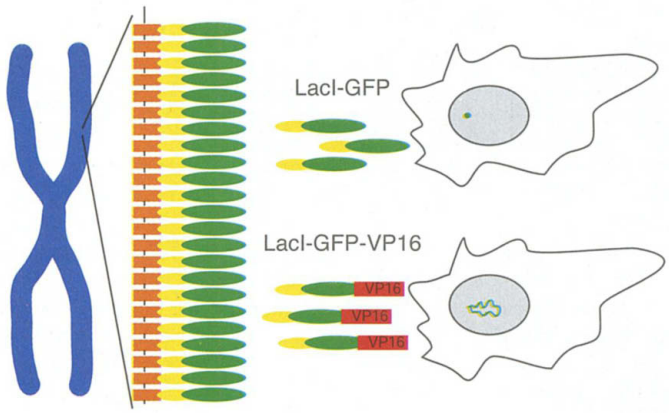

Fig. 2. Transcriptional activators induce large-scale changes in chromatin folding. (**Left**) DNA constructs containing LacI-binding sequences (orange boxes) upstream of a dihydrofolate reductase (DHFR) gene (navy blue) were integrated into chromosomes as tandem ~90-Mbp arrays by Tumbar and co-workers (*32*). (**Right**) This locus can be visualized in vivo by expressing a LacI-GFP fusion protein (yellow-green protein), which images as a single green focus within the nucleus (top). In contrast, expression of a LacI-GFP-VP16 activator protein (yellow-green-red protein) produced a ribbonlike chromonema fiber within a subset of cells (bottom). On the basis of comparison of ribbon length with the known base pair length of the DNA tract, fiber width was estimated at ~100 nm, which is considerably larger and more compact than even a fully condensed 30-nm nucleosomal array.

Fiber Heterogeneity: Specialized Domains of Chromatin Folding

Throughout this review, we have described chromatin as a linear array of canonical nucleosomes that undergoes regulated condensation-decondensation reactions. However, what we tend to forget is that an in vivo chromatin fiber is an extremely heterogeneous nucleoprotein filament (Fig. 3). First and foremost, in addition to canonical nucleosomes, in vivo chromatin arrays also contain novel types of nucleosomes that harbor one or more histone variants. For instance, nucleosomes assembled at yeast and mammalian centromeres contain a histone H3 variant, Cse4/CENP-A, which is essential for centromere function or assembly (*34*, *35*). Likewise, H2AZ (also known as H2A.Z or H2AvD) is a variant of histone H2A that contains NH_2- and COOH-terminal domains whose sequences are substantially different from that of canonical histone H2A (*36*). Studies in *Drosophila* and budding yeast show that H2AZ is widely, but not uniformly, distributed along chromosomes and that nucleosomes that harbor H2AZ are interdigitated with canonical nucleosomes (*37*, *38*). H2AZ is required for one or more essential roles in chromatin structure that cannot be replaced by bona fide histone H2A (*36*, *38*, *39*). In yeast, at least one of these essential roles is in transcriptional regulation, because H2AZ is required for both transcriptional repression (*40*) and activation (*41*).

In most cases, how histone variants alter nucleosome structure or change the folding properties of nucleosomal arrays is unknown. Nor is it known how these variant nucleosomes are localized to specific DNA sequences (for example, why are CENP-A–containing nucleosomes found only at centromeres?). One appealing possibility is that the targeted assembly of nucleosomes that harbor histone variants creates novel chromatin domains that have distinct folding properties. In the case of H2AZ, recent biochemical analyses with model 208-12 nucleosomal arrays support a specialized role for this variant in chromatin folding. Nucleosomal arrays containing H2AZ readily fold to 30-nm-like fibers, yet fail to self-associate even at high concentrations of divalent cations (*42*). Thus, chromatin enriched for this variant may only partially compact, resisting chromonema fiber formation and thereby facilitating transcription (Fig. 3).

In addition to nucleosomes, the chromatin fiber contains an enormous diversity of other accessory proteins. Like the core histones,

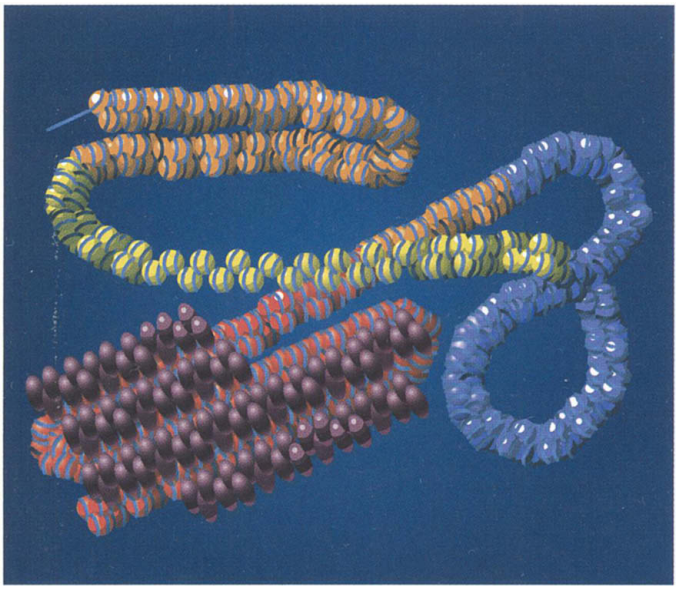

Fig. 3. Chromatin fiber heterogeneity influences the structural characteristics of chromatin. Differences in histone modifications or histone composition result in distinct structural consequences for localized domains. High levels of acetylation (yellow nucleosomes) favor fiber decondensation, with low levels of acetylation or linker histone association allowing more extensive condensation and fiber-fiber association (orange chromatin segments). In contrast, deacetylated, methylated regions (red regions) recruit nonhistone proteins such as HP1 (purple) that nucleate specialized structural domains such as centromeric heterochromatin. Incorporation of the histone variant H2AZ (blue nucleosomes) produces regions of chromatin fully condensed to a 30-nm fiber, unable to undergo intermolecular fiber-fiber condensation.

there are numerous linker histone variants that are nonrandomly incorporated into chromatin and expressed in developmentally restricted and cell type–specific patterns (*43*). There is also an abundance of nonhistone proteins, such as high mobility group–1 (HMG1), which are present at a ratio of about one per two nucleosomes (*44*, *45*). Other HMG proteins, such as HMG14 and HMG17, are known components of "active" chromatin (*46*) that bind to nucleosomes with high affinity and might disrupt higher order chromatin structure (*47*).

More recently, posttranslational modification of the histone tails has also been shown to alter the composition of the chromatin fiber. In particular, the chromodomain of *Drosophila* heterochromatin protein 1 (HP1), a principal component of constitutive heterochromatin, binds a histone H3 tail methylated at Lys^9 (*48*–*51*). The methylation of histone H3 Lys^9 is also highly enriched in pericentromeric heterochromatin (*52*, *53*) and at the inactivated X chromosomes of higher eukaryotic females (*54*, *55*), suggesting a broad role for this modification in the formation of facultative and constitutive heterochromatin. Although the structure of HP1-containing chromatin is not known, it is believed to be a highly condensed chromatin domain that excludes the transcription machinery. Thus, HP1 recruitment to site-specifically methylated chromatin likely creates inert, specialized domains of chromatin (Fig. 3) (*56*).

Conclusion

Rather than a simple linear array of nucleosomes, the chromatin fiber is composed of multiple specialized domains, each of which contains a distinct subset of canonical and variant nucleosomes, linker histone variants, and nonhistone proteins. We probably need to think of the complexities of the chromatin fiber in the same way as protein structure: Chromatin exhibits a primary structure (the linear arrangement of nucleosomes), a secondary structure (the 30-nm chromatin fiber), and a tertiary structure (chromonema-like fibers). Likewise, each level of structural complexity has the capacity to be independent of the others. For instance, extended domains of 30-nm fiber could exist between larger domains of thick chromonema fibers. Furthermore, transcriptional control may involve regulatory mechanisms that act at different levels of fiber structure. In this regard, we point out that the commonly employed nuclease digestion methods, such as deoxyribonuclease I or micrococcal nuclease, only monitor the primary structure of the chromatin fiber (that is, the structure of the extended fiber), not the more highly folded states. One of the major challenges of the future is to determine how different folded domains are established, how fiber heterogeneity alters chromatin structure, and how these changes in structure are interpreted by the transcription machinery. Clearly, the dissection of the mechanisms of transcriptional control on bona fide chromatin fibers awaits a biochemical system where such fibers can be accurately assembled and their folding dynamics fully characterized.

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