Chromatin remodeling in plants

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In the past two years, a variety of forward genetic screens have revealed predicted plant chromatin remodeling components that are involved in either differential histone acetylation or ATP-dependent SWI2/SNF2-related complexes. Combined with the results of recent reverse genetic studies, these findings have begun to provide the groundwork for determining the function of chromatin-based control in plants.

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Abbreviations

BRM	BRAHMA
CAF-1	chromatin assembly factor-1
CBP	CREB-binding protein
CHD	Chromodomain-Helicase-DNA-binding protein
DDM1	*DECREASED DNA METHYLATION1*
FAS1	*FASCIATA1*
HDA	histone deacetylase
HAT/HAC	histone acetyltransferase
PKL	PICKLE
SCR	*SCARECROW*
SWI/SNF	SWITCH/SUCROSE NON-FERMENTING
SYD	*SPLAYED*
TSA	trichostatin A

Introduction

The phrase 'chromatin remodeling' is commonly used as a catchall to describe the reconfiguration of protein–DNA interactions that accompany or potentiate changes in genomic activity (e.g. gene expression or recombination) [1]. Chromatin remodeling encompasses a diverse array of mechanisms, which are beginning to be defined — largely through genetic and biochemical studies in fungi and animals (see [2,3]). In this review, we explore recent work in plants concerning two of the best-understood chromatin remodeling mechanisms: first, differential core histone acetylation, and second, the action of ATP-hydrolyzing protein complexes. In addition, we discuss the interaction of chromatin remodeling and cytosine methylation, the most basic level of modification superimposed on eukaryotic genomes as epigenetic information. In the process, we consider the novel contributions of both forward and reverse genetics studies in plants connecting chromatin remodeling to gene silencing and development.

Plant chromatin

Plant chromatin organization closely resembles that of other organisms, being based upon the packaging of approximately 145 base pairs of DNA into core nucleosomes. Like many multicellular eukaryotes, plants express a diverse repertoire of genes encoding the core histones H2A, H2B, H3 and H4 (e.g. *Arabidopsis* has 45 core histone genes; URL www.chromdb.org), as well as multiple linker histones. The positions of nucleosomes surrounding the upstream regions of particular plant genes, and the nuclease accessibility of such regions, have been shown to change in response to environmental and developmental cues [4,5]. The importance of regulating chromatin in plants is highlighted by the developmental phenotypes of plant mutants with defective homologs of the *Drosophila* polycomb group proteins (e.g. *clf* [*curly leaf*], *fie* [*fertilization-independent endosperm*] and *medea* in *Arabidopsis*) [6–9]. In this review, our attention is placed on proteins and mechanisms involved in remodeling plant chromatin.

Plant histone acetylation

Core histones are subject to post-translational modifications, including acetylation, phosphorylation, and methylation [10]. The most-studied histone modification is the acetylation of conserved lysine residues, primarily in their amino-terminal tails. Histone acetylation levels are determined by the competing action of histone acetyltransferases (HAT or HAC) and histone deacetylases (HDAs). Elevated acetylation of lysines in the core histone tails is often associated with increased gene activity [11]. This association is not absolute, however. A number of genes are repressed in yeast mutants that have increased histone acetylation [12], contrary to the simple expectation that histone acetylation creates a more 'open' active chromatin configuration. How differential histone acetylation modulates chromatin changes is not clear, but the nucleosome crystal structure suggests that non-acetylated histone tails are free to interact with neighboring nucleosomes and mediate higher-order chromatin packaging [13].

Core histones are also reversibly acetylated in plants [14•]. A number of observations point toward the importance of histone acetylation in the biology of plants. One intriguing hint comes from the discovery of HC toxin, which is produced by a maize fungal pathogen, *Cochliobolus carbonum*. The toxin, which is required for pathogenesis in this system, specifically inhibits histone deacetylases in the plant host [15]. Mimicking this natural system, investigators have examined the effects of applying histone deacetylase inhibitors, such as trichostatin A (TSA) or butyrate, to whole plants or plant cells in culture. Application of these agents leads to hyperacetylation of nuclear proteins [16,17], but the effects of histone deacetylase inhibitors on plant physiology, development and gene expression programs remain poorly defined. Alteration of plant gene expression in response to the application of histone deacetylase inhibitors has been documented in only a few publications. In one notable example, TSA treatment of allopolyploid *Brassica napus*

(a diploidized *B. oleracea* × *B. rapa* hybrid) reactivated the quiescent ribosomal RNA genes originating from the *B. oleracea* parent [16].

Recent advances have begun to define tools that allow the dissection of the regulation and function of plant histone acetylation with more precision than that afforded by inhibitors. The biochemical characterization of several distinct forms of histone acetyltransferases and histone deacetyltransferases in maize has led the way [14•]. The most surprising finding of these studies was the identification of a novel class of nucleolar histone deacetylases, defined by the maize HD2 (HISTONE DEACETYLASE2) gene product [18]. Complementing the biochemistry, genetic tools to dissect histone acetylation are being assembled in *Arabidopsis*. The complexity of the machinery employed to modulate plant histone acetylation is highlighted by the large complement of 12 HAC genes and 15 HDA genes in the *Arabidopsis* genome (URL www.chromdb.org).

The characterization of specific plant histone acetyltransferases is just beginning. Like other eukaryotes, plants have two major classes of histone acetyltransferase enzymes: HAT-A and HAT-B [14•]. Most attention is focused on the HAT-A enzymes because they operate in the nucleus to acetylate histones that are incorporated into chromatin, and are most likely to be involved in controlling gene expression. Plant HAT-A enzymes, including *Arabidopsis* homologs of the transcriptional co-activators GCN5 [19••] and p300/CREB-binding protein (CBP) [20], are beginning to be characterized. Recombinant versions of *Arabidopsis* HAC1 (p300/CBP class) and GCN5 possess histone acetyltransferase activity. *Arabidopsis* GCN5 is capable of interacting *in vitro* with *Arabidopsis* orthologs of the yeast HAT-adaptor protein ADA2 [19••]. Moreover, *in vitro* data suggest that *Arabidopsis* ADA2 and GCN5 may be recruited to cold- and dehydration-inducible promoters by the C-repeat/DRE binding factor 1 (CBF1) transcription factor [19••]. Although these interactions remain to be demonstrated in plants, they represent the most detailed picture of how histone acetylation may be involved in a specific, environmentally induced gene expression program.

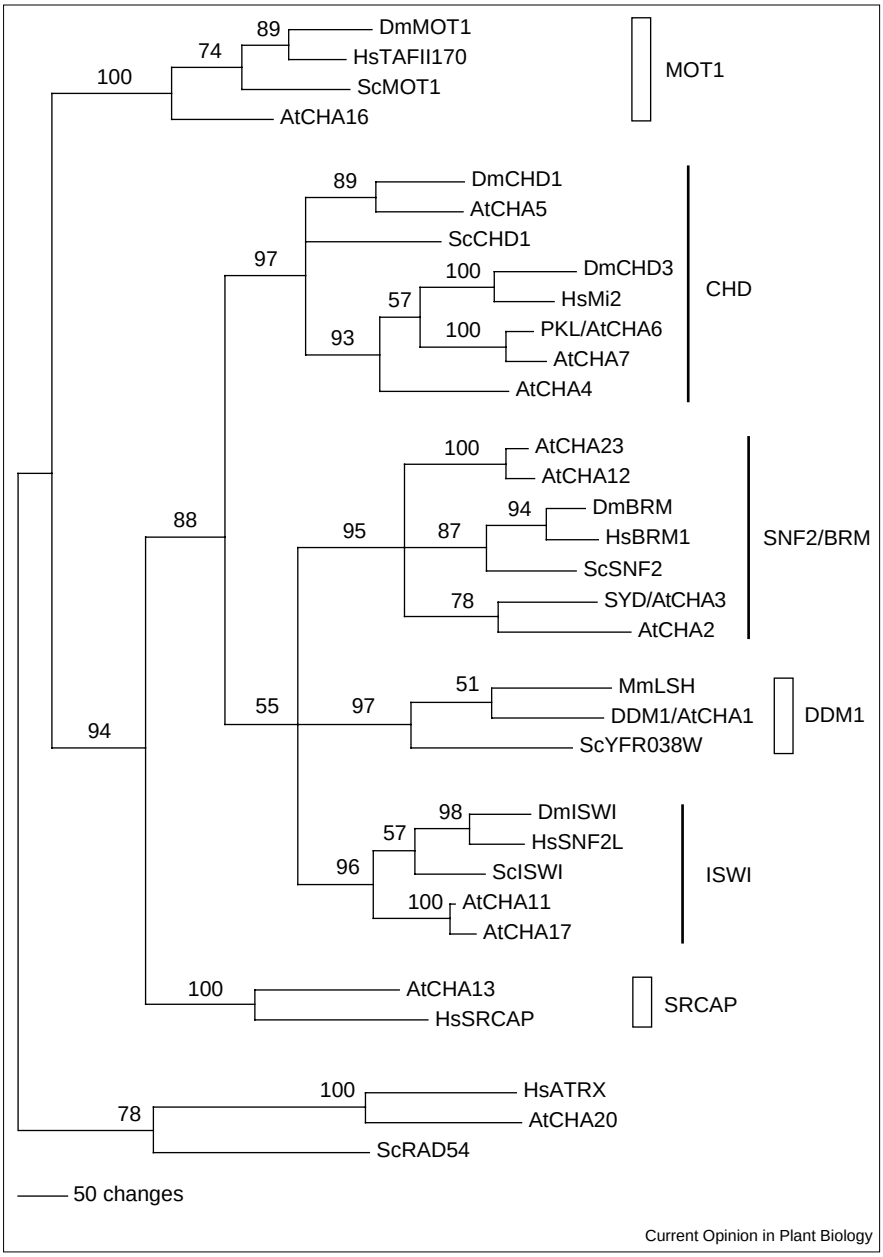
Overshadowing local changes in histone acetylation are the global shifts in histone acetylation that oscillate with the cell cycle. Using antibodies recognizing acetylated histone isoforms, Schubert and colleagues [21,22] have shown that the major changes in plant histone H4 acetylation detected at the cytological level are correlated with DNA replication rather than with transcriptional activity. The second class of histone acetyltransferases, the HAT-B enzymes, is responsible for these major cell cycle oscillations in histone modification. HAT-B enzymes primarily acetylate free cytoplasmic histone H4 (and possibly H3) before nuclear import and deposition into newly replicated chromatin. The best characterized plant HAT-B enzyme was recently purified from maize in a complex with a protein related to RbAp48 (the retinoblastoma-susceptibility-associated protein of 48 kDa), a WD40-repeat-containing protein that is associated with other chromatin modifying complexes [23].

Although work on plant histone acetyltransferases has proceeded largely along biochemical lines, the study of plant histone deacetylases has relied on genetic approaches. The expression of *HDA3*, one of four *HD2*-class genes in *Arabidopsis*, was suppressed by antisense expression, leading to stunted siliques and decreased seed set [24]. *HDA3* is primarily expressed in young siliques and flowers, prompting Wu *et al.* [24] to suggest that HDA3 is involved in the control of seed development. Although HDA3 inhibited gene transcription when directed to a specific promoter by *in vivo* tethering experiments, endogenous downstream targets of HDA3 have yet to be identified [24].

Transgenic *Arabidopsis* expressing antisense transcripts to *HDA1* (originally denoted *AtHD1* or *AtRPD3A*) have been reported by two groups [25,26•]. One of these two groups demonstrated that their antisense *HDA1* lines had a ten-fold increase in tetra-acetylated H4 histones [26•]; and that *HDA1*-antisense plants exhibited pleiotropic phenotypes, including delayed flowering time, serrated leaves, early senescence, homeotic changes, and floral abnormalities. The molecular basis for these phenotypes was not determined; however, one tissue-specific gene, the floral *SUPERMAN* (*SUP*) gene, was ectopically expressed in leaves of antisense-*HDA1* plants [26•], leading to the suggestion that aberrant developmental gene expression could be responsible. Traditional forward genetics netted another histone deacetylase gene, *AXE1* (*AUXIN GENE EXPRESSION1*) (*HDA6*), in a screen for mutations that increased the expression of a multiple copy auxin-responsive transgene in *Arabidopsis* [27•]. Mutants in *HDA6* exhibited increased expression of the auxin-responsive transgene, both in the presence and absence of auxin. Interestingly, none of the endogenous auxin-response genes tested were affected in *hda6* mutants, suggesting that HDA6 is involved primarily in modulating gene silencing rather than in controlling developmental gene expression.

The studies described above have yielded exciting clues, yet the central question remains: what is the function of histone acetylation in plant cells? The data in hand for plants could be used to argue for histone acetylation's role in orchestrating reversible developmental gene expression patterns. However, an equally plausible explanation is that histone acetylation levels are more important for 'locking-in' stable gene expression states to be inherited over many generations. The situation in the plant histone acetylation field at present, resembles the state of the plant DNA methylation field a few years ago. A reduction in DNA methylation in *Arabidopsis* leads to developmental abnormalities [28–30]. The defects are due to a combination of inherited epimutations and traditional genetic mutations that accumulate in DNA hypomethylation backgrounds, rather than to a breakdown

Figure 1



Predicted *Arabidopsis* SWI2/SNF2 chromatin remodeling proteins. A phylogeny was constructed using maximum parsimony optimization including all 39 predicted SWI2/SNF2 proteins in the *Arabidopsis* proteome, as well as selected animal and yeast SWI2/SNF2 proteins representing previously defined SWI2/SNF2 subfamilies ([37]; URL www.tigr.org/~jeisen/SNF2/snf2.html). For clarity, the tree shown focuses on the *Arabidopsis* SWI2/SNF2 proteins (AtCHA) that group unambiguously with the chromatin remodeling subfamilies SNF2/BRM, ISWI and CHD (subfamilies indicated on the right). The tree also includes three strongly supported subfamilies implicated in related functions: the MOT1 subfamily, which are involved in transcriptional co-repression by disruption of TATA binding-factor–promoter interaction; the SRCAP subfamily, which are associated with a CBP-type histone acetyltransferase [64]; and the DDM1 subfamily (see text). Representatives of the ATRX/RAD54 DNA repair subfamily were used to root the tree. Bootstrap values are shown; branches with bootstrap values of less than 50 were collapsed. See URL www.chromdb.org for more information and a comprehensive AtCHA phylogeny. At, *Arabidopsis thaliana*; Dm, *Drosophila melanogaster*; Hs, human; Mm, mouse; Sc, *Saccharomyces cerevisiae*.

in reversible developmental gene expression programs [29,31–33]. It will be important to determine whether the developmental abnormalities that arise in histone deacetylation mutants can be inherited, and whether they are stable in the absence of the antisense transgene or *hda* mutations. In addition, expression changes in both genes and non-genic sequences should be cataloged in histone acetylation mutants. Such data would allow the evaluation of the contributions of histone acetylation to the control of reversible developmental gene expression compared to its role in stabilizing inherited epigenetic states (e.g. in the silencing of transposons and heterochromatic repeats, and by cementing epialleles of developmental genes).

ATP-dependent chromatin remodeling in plants

An alphabet soup of chromatin remodeling machines capable of disrupting and reordering nucleosome–DNA interactions has been biochemically defined in *Drosophila*, yeast and mammalian cells (e.g. SWITCH [SWI]/SUCROSE NON-FERMENTING [SNF], CHRAC [chromatin accessibility complex], and NURF [nucleosome remodeling factor]) [2]. The engines of these remodeling machines are DNA-dependent ATPases in the SWI2/SNF2 superfamily [34,35]. In many cases, the chromatin remodeling machines function as transcriptional co-activators by facilitating access of transcription factors to DNA packaged in nucleosomes. However, some remodeling complexes exert a negative effect on transcription or are dedicated to other

processes, such as chromatin assembly. Kingston and Narlikar [36] have stressed the perspective that chromatin remodeling increases the fluidity of chromatin, lowering the activation energy between alternative chromatin states.

All of the eukaryotic genomes examined contain a number of SWI2/SNF2 family members, which can be grouped into different subfamilies on the basis of functional information and molecular phylogenetics [37]. At least three of the subfamilies (namely SNF2/BRAHMA [BRM], ISWI [Imitation Switch] and Chromodomain-Helicase-DNA-binding protein [CHD]) contain proteins with demonstrated chromatin remodeling activity, whereas most of the other subfamilies appear to be dedicated to various types of DNA repair. The *Arabidopsis* proteome contains 39 proteins that have the seven diagnostic SWI2/SNF2 motifs (URL www.chromdb.org). Several other *Arabidopsis* proteins contain one or more SWI2/SNF2 signature domains, but not the complete set of seven. Among these is MORPHEUS' MOLECULE 1 (MOM1), a protein required for gene silencing, which contains only domains IV, V, and VI [38]. Twelve of the *bona fide Arabidopsis* SWI2/SNF2 proteins group unambiguously with the chromatin remodeling subfamilies (Figure 1). Although molecular phylogenetics provides some hints about function, it is important to stress that none of the plant SWI2/SNF2 proteins have been demonstrated to possess remodeling activity either *in vitro* or *in vivo*.

Fortunately, some functional information is emerging from the genetic analysis of a handful of the putative plant SWI2/SNF2 remodeling proteins. One of the best examples is the genetic analysis of the *Arabidopsis* PICKLE (PKL) protein, a member of the CHD subfamily [39•,40•]. *Drosophila* and mammalian members of this subfamily act in complexes with histone deacetylases to repress genes [41,42]. Mutations in the *PKL* gene were identified by a variably penetrant 'pickle root' phenotype, which is caused by ectopic expression of embryonic developmental programs in the roots [43]. Another group identified *pkl* mutations (also called *gymnos*) as enhancers of the defective carpel phenotype conditioned by the *crabs claw* (*crc*) mutation [39•]. The mutant phenotypes, and the protein similarities, suggest that PKL articulates developmental programs by repressing genes in tissues where, or at times when, they are not necessary. At least one candidate target for *PKL* action has been identified. *LEAFY COTYLEDON 1* (*LEC1*), a global transcription factor responsible for the activation of a number of embryonic genes, is ectopically expressed in adult tissues of *pkl* mutants [40•].

Mutations in the *Arabidopsis SPLAYED* (*SYD*)/*CHROMATIN REMODELING COMPLEX SUBUNIT A 3* (*CHA3*) gene, which encodes a SNF2/BRM chromatin remodeling subfamily member, were found in a screen for enhancers of a weak *leafy* allele. *syd* mutants display pleiotropic developmental phenotypes, including precocious transition of the inflorescence meristem to floral meristem under certain environmental conditions (D Wagner, EM Meyerowitz, personal communication).

Pleiotropic developmental abnormalities have also been seen in *Arabidopsis* plants that are deficient in other proteins implicated in chromatin remodeling. *Arabidopsis* transgenics expressing an antisense transcript to *BUSHY* (*BSH*, also known as *CHROMATIN REMODELING COMPLEX SUBUNIT E 1* [*CHE1*]), a *SNF5* homolog, are bushy and sterile [44]. The SNF5 protein is a conserved component of SWI2/SNF2-associated chromatin remodeling machines, which is necessary for complex assembly in yeast [45]. Recently, the *Arabidopsis FASCIATA1* (*FAS1*) and *FAS2* genes were shown to encode two of the three protein subunits of chromatin assembly factor-1 (CAF-1) [46••]. The CAF-1 complex acts as a H3/H4 histone tetramer chaperone and facilitates chromatin assembly after DNA replication [47]. CAF-1 mutants in yeast are viable, but are defective in the maintenance of gene silencing at telomeres and mating-type loci [48,49]. Loss of epigenetic gene regulation has also been implicated as the cause of the developmental phenotypes of the *Arabidopsis fas* mutants [46••]. The normally tightly restricted spatial pattern of *WUSCHEL* (*WUS*) and *SCARECROW* (*SCR*) gene expression in shoot and root apical meristems, respectively, is disrupted in *fas* mutants. *SCR* expression is normally restricted to a small group of meristem cells and a single endodermal cell-layer extending toward the shoot. In *fas1* mutant root apices, however, *SCR* expression is sometimes lost completely in small sectors or is expressed ectopically in cells adjacent to those that normally express *SCR*. This stochastic pattern of *SCR* expression in *fas1* mutants led Kaya *et al.* [46••] to propose that *Arabidopsis* CAF-1 is necessary for proper epigenetic inheritance of gene expression states in daughter cells.

The results gathered to date for *pkl*, *syd*, *snf5*, and *fas* mutants point toward the importance of chromatin remodeling and assembly in the control of plant development, particularly in reference to the external environment, through maintenance of epigenetic gene-expression states. At the moment, only a handful of target genes affected by these mutations have been identified. Important next steps will include the definition of more target genes and the elucidation of the epigenetic mechanisms overseeing the regulation of these targets.

Interactions between chromatin remodeling and DNA methylation

Methylation of cytosine residues has been implicated in the control or reinforcement of epigenetic gene expression states for a number of years. The mechanisms by which DNA methylation can influence gene expression are now becoming clearer through recent work connecting cytosine methylation and chromatin remodeling [50]. An important breakthrough was the demonstration that mammalian methylcytosine-binding proteins can recruit histone deacetylase complexes [51–55]. In this way, inherited

cytosine methylation can act as a template for altering the level of local histone modification. In turn, chromatin structure may influence the level of local DNA methylation, as was shown by TSA-induced regional hypomethylation in *Neurospora* [56]. DNA methylation and chromatin may also be coordinated through the recently documented physical interaction between histone deacetylases and cytosine methyltransferases [57–59].

Work on chromatin remodeling in plants has provided additional clues to the importance of communication across the chromatin–methylation interface. For example, in the allopolyploid *Brassica napus* study mentioned earlier, application of the cytosine methylation inhibitor 5-azadeoxycytidine reactivated the silenced rRNA genes, with similar efficacy as TSA [16]. In this study, 5-azadeoxycytidine and TSA did not act synergistically to reactivate the quiescent rRNA genes, suggesting that histone acetylation and DNA methylation operate on a single pathway. Supporting the action of histone acetylation downstream of DNA methylation on a single pathway, antisense expression of *Arabidopsis HDA1* did not lead to detectable changes in genomic methylation levels, despite a 10-fold elevation in histone H4 acetylation [26•]. In contrast, butyrate treatment of *Petunia* has been reported to induce transgene promoter methylation [60]. Clearly, much remains to be understood about the relationship between histone acetylation and DNA methylation [61].

ATP-dependent chromatin remodeling also appears to interact with the DNA methylation system. Mutations in the *Arabidopsis* SWI2/SNF2 gene, *DDM1* (*DECREASED DNA METHYLATION1*; see Figure 1), lead to a rapid loss of cytosine methylation throughout the repetitive DNA component of the *Arabidopsis* genome, and to a more gradual depletion of methylation in low copy sequences [62•]. DDM1 may affect methylation indirectly by building chromatin architectures (e.g. heterochromatin) that are targeted by methyltransferases. Alternatively, DDM1 may be directly involved in providing methyltransferase access to hemimethylated DNA in the chromatin environment immediately after DNA replication. The DDM1 results are supported by the discovery that patients suffering from X-linked alpha-thalassemia/mental retardation (ATRX) syndrome, which is caused by a defective SWI2/SNF2 protein in a DNA-repair subfamily (see Figure 1), also exhibit alterations in DNA methylation (i.e. both hypo- and hyper-methylation) [63].

Conclusions

This brief review touches upon emerging areas in the study of chromatin remodeling in plants. Although the biochemistry of plant chromatin remodeling remains in its infancy, an expanding set of chromatin modification mutants promises to make plants important experimental systems for the study of chromatin-mediated regulation at the whole-organism level. In addition, plants offer a diverse palette of epigenetic phenomena and developmental plasticity with which to gauge the effects of manipulating chromatin. Another important consideration is that available mutations disrupting chromatin remodeling in plants are viable, whereas many of their animal counterparts cause severe phenotypes or are lethal. This may reflect a larger degree of genetic redundancy in plants relative to animals, or may result from plants' higher tolerance of perturbation in gene expression and dosage (witness the general viability of aneuploids in plants as opposed to animals).

The challenge in the near future will be to work out the biochemistry of plant chromatin remodeling proteins/complexes and to identify plant genes that are regulated via differential chromatin states. Forward genetics will likely continue to connect more components of chromatin remodeling machinery to interesting developmental phenotypes. Reverse genetics also will play an important role in this analysis. A National Science Foundation (NSF)-funded consortium is working to disrupt a large number of genes involved in chromatin metabolism in *Arabidopsis* and maize (URL www.chromdb.org). We look forward to seeing these tools exploited by the community to investigate the role of chromatin in the development, environmental interaction, life history, epigenetics, and evolution of plants.

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