Immediate early genes (IEGs) belong to a class of genes that are rapidly activated, usually in a transient fashion, in response to intracellular signaling cascades. The induction of IEGs occurs in the absence of de novo protein synthesis and thus cannot be blocked by protein synthesis inhibitors.

IEGs encode for secreted proteins, chemo-attractants, cytoplasmic enzymes, ligand-dependent transcription factors and inducible transcription factors (ITFs), comprised of the Fos, Jun and Krox protein families. In this review, we narrow our scope to the c-fos and c-jun IEGs belonging to the Fos and Jun families. Fos and Jun oncoproteins contain a bZIP region consisting of a basic DNA-binding domain and a leucine zipper domain, and together they form dimeric complexes that stimulate transcription of genes containing AP-1 regulatory elements. Jun was originally thought to be identical to the transcription factor AP1, but now it is known that AP1 is not a single protein but constitutes a group of related dimeric basic region-leucine zipper proteins that belong to the Jun, Fos, Maf and Atf subfamilies. Fos can heterodimerize with Jun to form AP-1 transcriptional factor whereas Jun acts on SRE. The promoter is organized into the serum response element (SRE), c-Sis inducible element (SIE), TCF (ternary complex factor), a calcium response element (CRE) and AP-1/CRE regulatory elements. It is located on chromosome 12q24.3-31. The four-exon structure of the c-fos gene was isolated in 1982 from Finkel-Biskis-Jinkins murine osteogenic sarcoma virus and shortly thereafter, its cellular counterpart, c-fos was described. c-fos is known to be a nuclear phosphoprotein that forms heterodimer with mainly c-jun transcription factor family and stimulates genes that contain AP-1 regulatory elements. It is located on chromosome 12q24.3-31. The four-exon structure of the c-fos gene is highly conserved among species, spans approximately 4kb and encodes for a 380 amino acid protein of size 55 to 62 kDa.

The promoter is organized into the serum response element (SRE), c-Sis inducible element (SIE), TCF (ternary complex factor), a calcium response element (CRE) and AP-1/CRE as shown in Fig. 1. SRE is involved in the induction of c-fos in response to growth, phorbol esters and ionizing stimulants, sometimes but not always, via PKC-dependent pathways. SRE contains a CCA/T-GG sequence or the CARG box, and serum response factor (SRF) and its accessory protein, TCF/Elk-1 bind to CARG box of the SRE; these transcriptional factors can be subjected to serine phosphorylation through c-fos transcription can be repressed by AP-1 acting on SRE. SIE is a direct repeat element which binds STATs and TFII-I that respond to extracellular signaling through serine phosphorylation. CREB protein, AP-1 dimers and Ca2+-dependent cAMP stimulate the transcription of c-fos via CRE.

Expression of c-fos is more tran-
sient than c-jun as it degrades faster due to two factors: the instability of its transcript in the AU-rich element (ARE) in the 3′ untranslated region and a sequence in the protein-coding segment. c-fos expression exhibits a biphasic decay: the first phase, which has a half-life of 45 min, when 20—30% of c-fos molecules are bound with Jun proteins; the second phase, which has a half-life of 90—120 min, occurs when c-fos synthesis is stopped and with 90% of all c-fos molecules associated with Jun proteins (Herdegen et al. 1998), and references therein). The formation of c-fos: c-jun dimers, in return, promotes the degradation of c-fos. 2

The c-jun Gene and Its Promoter Jun is a putative oncogene of avian sarcoma virus 17, with homologues in several other vertebrate species. It was discovered by Y. Maki et al. in 1987 who gave it the name Jun, the Japanese number 17 or ‘ju-nana’, followed with the discovery of its cellular counterpart, c-jun.11) c-jun is mapped in the p32-33 region of chromosome 1, an intronless gene11) and encodes for a 334 amino acid of 55 kDa.

The organization of the c-jun promoter contains DNA binding elements NF-jun (nuclear factor-jun, with homology to NF-kB), Sp1 binding sites, the CCAAT-box, jun1 and jun2 (Fig. 2). The c-jun promoter is regulated at the jun1 site by the binding factors such AP-1, pCREB, c-jun dimers. jun2 site is another AP-1 site that binds to Jun:ATF-2 heterodimers for autoregulation of its own gene and can either work independently or together with jun1.

Histones associate extensively with the DNA in the nuclei of eukaryotic cells. Collectively, an octamer of highly conserved histone proteins (two H2A-H2B dimers and an H3-H4 tetramer) wrapped around in 1.75 turns of 146 bp DNA, an assembly known as a nucleosome.15,16) Nucleosomes are connected to another nucleosome with 10—90 bp of linker DNA17) and stabilized by the binding of a fifth histone, H1 to each nucleosome and to its adjacent linker.18) There is further folding of the chromatin fibre into higher-order structures so as to package eukaryotic genomes within the nuclei.

Each core histone is composed of a structured domain and an unstructured amino-terminal tail of varying lengths from 16 amino acids for H2A, 32 residues for H2B, 44 amino acids for H3 and H4 with 26 amino acids.19,20) These histone tails are protease sensitive and comprise 25—30% of the mass of individual histones. When subjected to covalent modification by a variety of cellular factors, they are proposed to act as signals from the DNA to the cellular machinery for various processes including transcription, chromosomal condensation and mitotic condensation.21,22)

The most compact form of chromatin is called heterochromatic or ‘off’ state and is inaccessible to regulatory signals entering the nucleus. To achieve an euchromatic or ‘on’ state, the chromatin has to unpack to expose important cis regulatory sequences such as enhancer-binding sites, the TATA box, or the transcription initiation site for transcriptional factors to bind to nucleosome-free regions of DNA.23) The chromatin structure can be altered by two main mechanisms: either by post-translational modifications of the aminotermi tails of histones or remodeling of nucleosomes via ATP-dependent chromatin remodeling complexes.24)

The core histone N-termini tails are susceptible to a wide range of covalent modifications including acetylation, phosphorylation, methylation and ADP-ribosylation22—25) (Fig. 3). Ubiquitination can occur on the C-termini tails of H2A and H2B. Only one modification can occur on one residue on a tail at a given time.

Histone Acetylation Histone acetylation is the best-characterized modification. Histone acetylation is a reversible modification of lysine residues within the N-termini.

**Fig. 2. The c-jun Promoter Organization**

The c-jun promoter is organized into the NF-jun (nuclear factor-jun), Sp1 binding sites, the CCAAT-box, jun1 and jun2. NF-jun site is an 11 bp sequence within the c-jun promoter and is regulated by NF-jun transcription factor, translocated from the cytoplasm to the nucleus when stimulated by NF-kB. Sp1 sites can be simultaneously bound by Sp1 transcriptional factors and the CCAAT-box is recognized by CCAAT-binding transcription factor or CTF. The c-jun promoter is regulated at the jun1 site by the binding factors such as AP-1, pCREB, c-jun dimers. jun2 site is another AP-1 site that binds to Jun:ATF-2 heterodimers for autoregulation of its own gene and can either work independently or together with jun1.

PACKAGING: HISTONES, NUCLEOSOMES AND CHROMATIN

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There are 26 sites of acetylation on a nucleosome and histone acetylation is dynamically regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) to achieve appropriate levels of transcription. HATs function enzymatically by transferring an acetyl-group from acetyl-coenzyme A (acetyl-CoA) to the ε-amino group of the lysine side chain within a histone's basic N-terminal tails, and HDACs act in the reverse to remove the acetyl group. HATs and HDACs function as multi-subunit complexes. Acetylation results in charge neutralization of the basic histone tails to weaken histone-DNA or nucleosome-nucleosome interactions for accessibility to gene locus. Though there is evidence that acetylation leads to transcription and deacetylation can cause repression, not all histone acetylation leads to activation of gene.

**Enzymes Involved in Histone Acetylation**

HATs are categorized into two classes, based on their suspected cellular origin and functions. Type B HATs, like Hat1, are located in the cytoplasm and play a housekeeping role of acetylating newly synthesized free histones in the cytoplasm prior to their assembly with newly-replicated DNA in chromatin, though these histones would have to be deacetylated by histone deacetylases before incorporation into the chromatin. Type A HATs are located in the nucleus and function to acetylate nucleosomal histones. Type A HATs are linked to transcription-related acetylation events consisting of GNAT superfamily (including Gcn5, PCAF and Elp3), MYST family, p300/CBP and the nuclear receptor coactivators SRC-1, ACTR, TIF2, TAFII 250 and TFIIC. At this point, it is interesting to note that HATs are not restricted to acetylating histones. HATs usually do not work in isolation but in complexes with SAGA and PCAF complex, NuA3 and NuA4 complex and MSL complex. Bromodomains in Type A HATs are said to target and bind to acetyl-lysines of histone tails, but their exact mechanism is still unknown. Their recruitment to histone tails could be due to the specific spacing between the lysine residues in the histone tails.
The two most widely studied HATs are the functional homologs, p300 and CBP (often referred to as p300/CBP) and they serve as coactivators interacting with sequence-specific transcriptional factors that target at acetylated portions of promoter regions. In 1996 the transcriptional coactivators p300 and CBP were found to have intrinsic HAT activity with their ability to acetylate all four core histones in nucleosomes and also free histones. p300/CBP and p300/CBP-associated factor (PCAF) form a complex on specific promoter elements via interaction with DNA-binding activators, like CREB, c-jun, c-Myb, nuclear hormone receptors, Sap-1a and MyoD to acetylate histone tails in a promoter-specific manner and disrupt the repressive chromatin structure.

Histone deacetylases (HDACs) are complementary enzymes of HATs, acting in a reverse manner to remove the acetyl group from the lysine groups in histones. The first deacetylase to be discovered was mammalian HDAC1 and other members of HDACs chromodomain protein, retinoblastoma protein-associated proteins and Sin3 were subsequently included. Together with a chromatin-remodeling factor (covered in the last portion of this review), NuRD/Mi-2, is recruited to the chromatin template by transcriptional repressors or histone methylation and facilitates the access of HDACs to histone tails to fold chromatin so as to repress transcription.

**Histone Methylation** Histone methylation is the least understood of the post-translational modifications of histones. The reasons for the ambiguity of this modification are that the enzymes known to be involved are still in their infancy, and electrophoretic resolution is difficult as methylation does not greatly affect the charge change on lysine and arginine as much as acetylation and phosphorylation. At this stage, it is known that histone methylation by histone methyltransferases (HMTases) affects the ε-amino groups of lysine (K) and arginine (R) residues of H3 and H4 tails. Figure 4 shows methylation of lysine residue.

Histone arginine methyltransferases or protein arginine methyltransferases (PRMTs) involves the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the guanidine nitrogens of arginine residues in mono- or di-methylation in either symmetrical (Type II PRMT) or asymmetrical (Type I PRMT) fashion. Examples of Type I PRMTs are H4-specific PRMT1, PRMT3, RMT1/HMT1 and H3-specific CARM1/PRMT4. PRMT1 methylates specifically at H4–R3 residue and CARM1/PRMT4 shows specificity in H3–R2, R17 and R26 residues. PRMT5 is a Type II methyltransferase and is able to methylate H2A and H4 in vitro, but studies have not validated if it can methylate them in vivo. Methyltransferases are recruited to promoters, acting as coactivators to induce transcription.

Histone arginine methylation is involved in gene activation whereas histone lysine methylation can either lead to gene repression or activation. Lysines may be mono-, di- or trimethylated at preferred lysine sites at histone H3 tails-K4, K9, K27, K36 and histone H4 tails-K20 by histone lysine methyltransferases containing SET-(Suvar3-9, Enhancer of Zeste, and Trithorax) or chromo-domain, which is highly conserved and flanked with cysteine-rich regions, and leading to gene silencing and transcriptional silent heterochromatin. For example, SUV39H1 (or Su(var)39–Suppressor of variegation) has intrinsic histone methyltransferase activity that causes H3–K9 methylation to recruit via the chromodomain of heterochromatin protein (HP1), leading to gene silencing by maintaining the heterochromatin state.

On the other hand, H3–K4 methylation is a mark of euchromatin in *Schizosaccharomyces pombe* and higher eukaryotes. Bernstein et al. (2002) also demonstrated that Set1 HMTs dimethylates H3–K4 and this protects active coding regions from deacetylation by occluding HDACs from binding to H3 tail, keeping the coding region in an acetylated active state.

In contrast with histone acetylation, which is dynamically and rapidly reversed by HDACs, histone demethylase is not yet characterized. This could be the reason why histone methylation is a relatively stable modification with slow turnover rate, and has been suggested as a heritable epigenetic mark to maintain chromatin states.

**Histone Phosphorylation** In recent years there has been explosion of identified signal transduction and mitotic protein kinases that phosphorylate H3–S10 residue and other serine (S) and histidine (H) residues on N-terminals of H2A-S1, H3-S28, H4-S1, -H18, H2B-S14, -S32 histone tails and on both N-terminal and C-terminal domains of H1 linker histone. Nuclear MSKs, RSKs that lie downstream of ERKs, yeast kinase Snf1 and Drosophila kinase, *Jil-1*. The phosphate donor could be a nucleotide triphosphate, ATP, GTP or cAMP. The modification can be reversed by protein phosphatases (PP).

Histone phosphorylation can result in both gene activation and repression. Phosphorylation of H1 and H3 are cell cycle dependent and the highest level of this modification occurs in the M-phase in which chromosome condensation is observed especially when linker H1 is phosphorylated, but it has been observed that chromosomal condensation does not require H1 hyperphosphorylation to occur. Likewise, the dual personality of H3–S10 phosphorylation in both transcriptional activation of immediate early genes and mitotic chromosome condensation further argues that this modification may not directly affect alteration of the chromatin structure but, more likely, phosphorylated residues are used as a binding surface for other transcription factors instead.

Histone phosphorylation on H3 could be that the addition of negatively charged phosphate groups may disrupt electrostatic interactions between basic H3 lysine-rich tails and the negatively charged DNA backbone to increase the accessibility of the genome to nuclear factors, just like the effects of histone acetylation on the chromosomal structure. However, it has been documented recently that...
Histone tails that are phosphorylated at H3–S10 during interphase tend to be more preferentially acetylated by HATs than unmodified H3 tails. An H2A variant in mammalian cells, H2A.X, is rapidly phosphorylated at its C-terminal Ser139 residue when exposed to ionizing radiation. This event has been termed as γ-phosphorylation and may possibly facilitate the access of the radiation-induced DNA double strand breaks to repair mechanisms (45 and references therein). H2B phosphorylation has also been reported during apoptosis as well, coinciding with the initiation of DNA fragmentation seen at early stages of apoptosis.

Histone Acetylation, Phosphorylation and Methylation in the Induction of IEGs

Phosphorylation appears to occur prior to and may be involved in promoting acetylation. Soloaga et al. (2003) identified two downstream nuclear kinases, MSK1 and MSK2 that phosphorylate histone H3 at Ser-10 and Ser-28 sites and nucleosome-binding high-mobility-group HMG-14 at Ser-6 site via the MAP-kinase pathway in primary mouse fibroblast. Another group showed there is increased Ser-10 H3 phosphorylation in Ras-MSAPK stimulated oncogene-transformed mouse fibroblast, identifying PP1 as the phosphatase responsible for phosphorylation. However, blocking H3 phosphorylation by H89, a MSK1 inhibitor, does not affect H3 acetylation or the induction of IEGs, c-fos and c-jun. In agreement with previous studies, only a subset of nucleosomes is phosphorylated on the H3 histone tail and inhibition of H3–Ser10 phosphorylation may not necessarily be synergistically coupled to H3 acetylation, and to a lesser extent, affecting downstream induction of IEGs.

In a recent paper by Martens et al. (2003), it was described that a dynamic recruitment of different factors and histone modifications are important for collagenase type I early gene activation. The binding of SET9 methyltransferase methylates histone H3 lysine 4 residue in a di-methylation and trimethylation fashion, followed by the assembly of a complex consisting of c-jun, c-fos, TATA binding protein and RNA polymerase II. Together with the assembly of the pre-initiation complex, an ordered binding of acetyltransferase p300 acetylates histones H3 and H4 and RSK2 kinase phosphorylates histone H3 serine 10. The chromatin remodeling factor SWI/SNF is then recruited to alter the structure of the promoter-bound nucleosome in an ATP-dependent manner to activate this gene.

Histone Code Hypothesis: An Interplay of Modifications

With the explosion of histone modifications, Allis and co-workers proposed a histone code hypothesis, whereby the pattern of modifications on one or more tails can result in downstream transcriptional events. Thus, the histone modifications work in concert and can affect neighboring residues, on either the same tail (in cis) or on different tails (in trans). One postulated mechanism of multiple modifications is that they might directly affect the chromatin structure or nucleosomes. The modifications can result in unique surface binding affinities for non-histone, chromatin-associated proteins like HMG, maybe through the charge patch changes on the histone tails and the modifications are interdependent in either a synergistic or antagonistic manner. If this truly exists, it means that we are able to predict a biological response by extrapolation of modified residues and its possible mediation of downstream functions.

For this purpose of mining histone modifications, chromatin immunoprecipitation (ChIP) assays (Fig. 6) have been developed to measure protein-DNA interactions with relation to the different functional states of the chromatin structure. Briefly, tissues or cells are treated with formaldehyde to crosslink proteins to DNA followed by sonication to fragment the chromatin. An antibody is applied against the protein of interest binding to the specific segment of chromatin, immunoprecipitating the chromatin fragments. Polymerase chain reaction of the immunoprecipitate with primers of the segment amplifies the signal and is used for the determination and analysis of the protein interacting with the site in the cells. Most histone modifications are determined by this highly sensitive method.
CHROMATIN REMODELING ENZYMES

Histone modifications are not the only means by which chromatin can be altered at promoters to regulate gene transcription. Another class of ATP-dependent chromatin remodeling enzymes is identified. ATP-dependent chromatin remodeling complexes are further divided into three groups based on the overall sequence similarity of their ATPase subunits to yeast Switch/Sucrose non-fermenting 2 (SWI2/SNF2 subfamily), *Drosophila* ISWI (also known as SNF2L) or human Mi-2 (CHD1 subfamily). All groups consist of a SWI2/SNF2-like ATPase domain but each has unique domains: SWI2 subclass contains a bromodomain, the ISWI subclass contains SANT domain and the Mi-2 subclass contains a chromodomains. SWI2 enzymes are stimulated by ‘free’ and nucleosomal DNA whereas ISWI and Mi-2 enzymes are optimally stimulated by nucleosomal DNA. The SWI2 class is known to cause transcriptional activation or repression, Mi-2 class enzymes and some members in the ISWI subclass have been shown to cause gene repression. RSC (Remodel for Structure of Chromatin) is a 15-protein ATP-dependent chromatin-remodeling complex related to SWI/SNF, the prototypical ATP-dependent nucleosome remodeler in budding yeast.

Chromatin remodeling factors use the energy derived from ATP hydrolysis to catalyze the rate at which the DNA structure interchanges, increasing the fluidity of the chromatin. They alter the nucleosome possibly by modifying the histone-DNA interface and often causing a net movement of a nucleosome relative to DNA, or nucleosome sliding. It was first proposed that SWI/SNF might disrupt nucleosome by dislodging H2A/H2B dimer but when H2A and H2B were crosslinked to prevent displacement, it did not prevent nucleosome disruption. However, histone-DNA disruption within H3/H4 tetramers alone was sufficient in SWI/SNF-assisted nucleosome disruption. SWI/SNF can cause nucleosome sliding by introducing superhelical torsion into nucleosomal DNA; this forms nucleosomes that contain exposed DNA bulges or loops whereby DNA could spontaneously unpeel from the ends to almost the center of the nucleosome causing <147 bp of DNA to associate with histone octamer to break histone-DNA contacts. Histones can also be physically transferred by RSC to an acceptor DNA. SWI/SNF and ISWI remodeled nucleosomal DNA are postulated to be more susceptible to nuclease digestion, thus creating the nucleosome-free regions for binding of transcriptional factors and polymerases.

In mitotic genes, SWI/SNF remodels a nucleosome by ATP-hydrolysis before GCN5-dependent histone acetylation and recruitment of transcriptional factors to promoter regions. The reason for this phenomenon could be that HAT complexes cannot penetrate closed chromatin without initial remodeling. However, for other, highly inducible cell cycle independent genes like IFN-β, histone acetylation appears to precede ATP-dependent remodeling, probably due to the fact that the chromatin is poised and opened. Yet, not all inducible cell cycle independent genes behave as such, for example, c-fos gene transcription is repressed by hSWI/SNF. Chromatin remodeling enzymes could exist just to increase the rate of conversion between different chromatin states, so that at an appropriate time, DNA could be accessed by regulatory processes.

CONCLUDING REMARKS

The histone modifications research field is exploding but is still in its infancy stage. So far, we observe modifications on histones during gene activation but it is still unclear on how each of this modification function is necessary or required for the transcription of a gene. In the case of the early response gene, histone acetylation and phosphorylation are observed during the induction of *c-fos* and *c-jun* though their exact function or purpose is unknown. However, such combinations of acetylation, phosphorylation and methylation may be code for recognition and binding by chromatin regulators, such as HATs or the remodeling of chromatin fiber or nucleosomes. Though we have only attempted to tell a portion of the tale of how a gene is transcribed by histone modification and their cross-interaction with another modification, these combinatorial modification codes might be the key to understanding the transcriptional regulation of immediate early genes.

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