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Review Article

ΔFosB: a Molecular Switch for Reward

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Abstract ΔFosB is a member of the Fos family of transcription factors. While all other family members are induced rapidly but transiently in response to a host of acute stimuli, ΔFosB is unique in that it accumulates in response to repeated stimulation due to its unusual protein stability. Such a prolonged induction of ΔFosB, within the brain's reward regions, has been implicated in animal models of drug addiction, with a wealth of evidence indicating that ΔFosB promotes reward and motivation and serves as a key mechanism of drug sensitization and increased drug self-administration. This has been validated in humans postmortem, with elevated ΔFosB levels seen in reward regions of the addicted brain. As a transcription factor, ΔFosB produces this behavioral phenotype by regulating the expression of specific target genes. We are identifying such transcriptional targets of ΔFosB by use of a candidate gene approach as well as by use of genome-wide methods. Recent work has analyzed chromatin remodeling—changes in the posttranslational modifications of histones and other nuclear proteins at drug-regulated genes—to delineate the detailed molecular mechanisms by which ΔFosB regulates target gene expression in vivo to mediate drug-induced synaptic, neural, and behavioral plasticity. These studies of ΔFosB are providing new insight into the molecular basis of drug addiction, which is defining a host of new targets for possible therapeutic development.

Keywords addiction; depression; resilience; chromatin remodeling; epigenetics; nucleus accumbens; drug abuse

1 Introduction

The study of transcriptional mechanisms of addiction is based on the hypothesis that regulation of gene expression is one important mechanism by which chronic exposure to a drug of abuse causes long-lasting changes in the brain that underlie the behavioral abnormalities that define a state of addiction [49]. A corollary of this hypothesis is that drug-induced changes in synaptic transmission and in neuronal excitability and morphology in particular brain regions, which have been implicated in addiction, are mediated in part via changes in gene expression.

Work over the past 20 years has provided increasing evidence for a role of gene regulation in drug addiction models, as several transcription factors—proteins that bind to specific responses elements in the promoter regions of target genes and regulate those genes' expression—have been implicated in these models. Prominent among these transcription factors is ΔFosB, a member of the Fos family of proteins. This review provides a progress report on ΔFosB, which appears to play a unique role in the addiction process, as a way to illustrate the types of experimental approaches that have been used to investigate transcriptional mechanisms of addiction.

2 Induction of ΔFosB in nucleus accumbens by drugs of abuse

ΔFosB is encoded by the *fosB* gene (Figure 1) and shares homology with other Fos family transcription factors, which include c-Fos, FosB, Fra1, and Fra2 [45]. These Fos family proteins heterodimerize with Jun family proteins (c-Jun, JunB, or JunD) to form active AP-1 (activator protein-1) transcription factors that bind to AP-1 sites (consensus sequence: TGAC/GTCA) present in the promoters of certain genes to regulate their transcription. Fos family proteins are induced rapidly and transiently in specific brain regions after acute administration of many drugs of abuse (Figure 2) (e.g., [18,23,86]). These responses are seen most prominently in nucleus accumbens (NAc) and dorsal striatum, which are important mediators of the rewarding and locomotor actions of the drugs. All of these Fos family proteins, however, are highly unstable and return to basal levels within hours of drug administration.

Very different responses are seen after chronic administration of drugs of abuse (Figure 2). Biochemically modified isoforms of ΔFosB (Mr 35–37 kD) accumulate within the same brain regions after repeated drug exposure, whereas other Fos family members show desensitization (i.e., reduced induction compared with initial drug exposures) [8, 11,21]. Such accumulation of ΔFosB has been observed for virtually all drugs of abuse (Table 1) (e.g., [24,34,42,44, 46,53,54,60]), although different drugs differ somewhat in the relative degree of induction seen in NAc core vs. shell and dorsal striatum [59]. At least for some drugs of abuse,

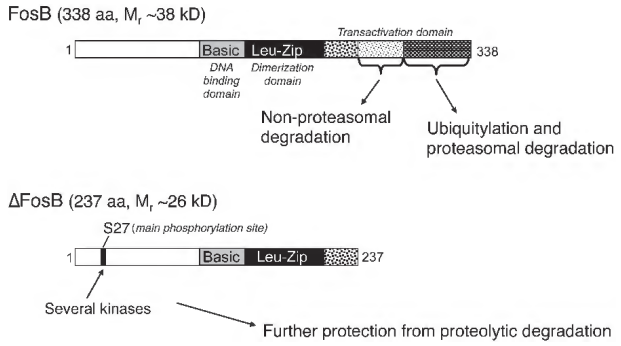

Figure 1: *Biochemical basis of ΔFosB's unique stability.* ΔFosB and FosB are encoded by the *fosB* gene. ΔFosB is generated by alternative splicing and lacks the C-terminal 101 amino acids present in FosB. Two mechanisms are known that account for ΔFosB's stability. First, ΔFosB lacks two degron domains present in the C-terminus of full length FosB (and found in all other Fos family proteins as well). One of these degron domains targets FosB for ubiquitylation and degradation in the proteasome. The other degron domain targets FosB degradation by a ubiquitin- and proteasome-independent mechanism. Second, ΔFosB is phosphorylated by several protein kinases at its N-terminus which further stabilizes the protein.

Table 1: Drugs of abuse known to induce ΔFosB in the NAc after chronic administration.

Opiates[1]
Cocaine[1]
Amphetamine
Methamphetamine
Nicotine[1]
Ethanol[1]
Phencyclidine
Cannabinoids

[1]Induction reported for self-administered drug in addition to investigator-administered drug. Drug induction of ΔFosB has been demonstrated for most drugs in both rats and mice. There are no reports of drugs of abuse that do not induce ΔFosB in NAc.

induction of ΔFosB appears selective for the dynorphin-containing subset of medium spiny neurons located in these brain regions, those neurons that predominantly express dopamine D1 receptors [32, 44, 46, 53], although more work is needed to establish this with certainty. Drug induction of ΔFosB in NAc has recently been demonstrated in humans [67].

The 35–37 kD isoforms of ΔFosB dimerize predominantly with JunD to form an active and long-lasting AP-1 complex within these brain regions [8, 22]. However, recent in vitro evidence has indicated that ΔFosB can also form homodimers with distinct physico-chemical properties compared to ΔFosB:JunD heterodimers [25]. An important focus of current research is to determine whether such

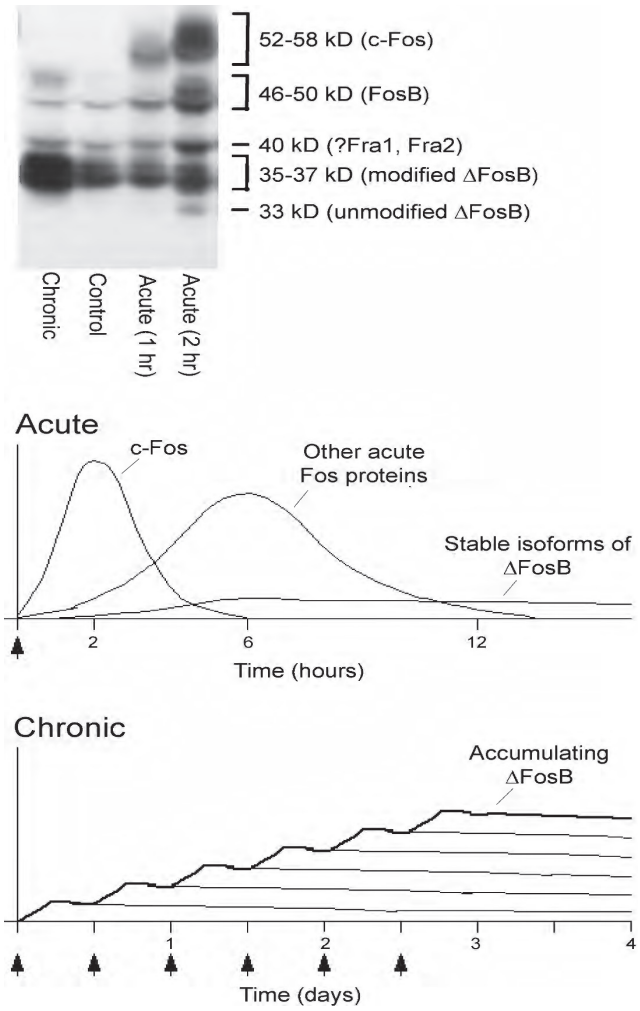

Figure 2: *Scheme showing the gradual accumulation of ΔFosB versus the rapid and transient induction of other Fos family proteins in response to drugs of abuse. Top:* the autoradiogram illustrates the differential induction of Fos family proteins in the NAc by acute stimulation (1–2 h after a single cocaine exposure) versus chronic stimulation (1 day after repeated cocaine exposure). *Bottom:* upper graph shows that several waves of Fos family proteins (comprised of c-Fos, FosB, ΔFosB [19, kD isoform], Fra1, Fra2) are induced in NAc and dorsal striatal neurons by acute administration of a drug of abuse. Also induced are biochemically modified isoforms of ΔFosB (35–37 kD); they are induced at low levels by acute drug administration, but persist in brain for long periods due to their stability. The lower graph shows that with repeated (e.g., twice daily) drug administration, each acute stimulus induces a low level of the stable ΔFosB isoforms. This is indicated by the lower set of overlapping lines, which indicate ΔFosB induced by each acute stimulus. The result is a gradual increase in the total levels of ΔFosB with repeated stimuli during a course of chronic treatment. This is indicated by the increasing stepped line in the graph.

Table 2: Behavioral phenotype upon ΔFosB induction in dynorphin/D1-type medium spiny neurons of NAc and dorsal striatum[1].

Stimulus	*Phenotype*
Cocaine	Increased locomotor responses to acute administration Increased locomotor sensitization to repeated administration
	Increased conditioned place preference at lower doses Increased acquisition of cocaine self-administration at lower doses
	Increased brain stimulation reward Increased incentive motivation in progressive ratio procedure
Morphine	Increased conditioned place preference at lower drug doses
	Increased development of physical dependence and withdrawal
	Decreased initial analgesic responses, enhanced tolerance
Wheel running	Increased wheel running
Sucrose	Increased incentive for sucrose in progressive ratio procedure
High fat	Increased anxiety-like responses upon withdrawal of high fat diet
Sex	Increased sexual behavior in males and females

[1]The phenotypes described in this table are established upon inducible overexpression of ΔFosB in bitransgenic mice where ΔFosB expression is targeted to dynorphin/D1-type medium spiny neurons of the NAc and dorsal striatum; several fold lower levels of ΔFosB are seen in hippocampus and frontal cortex. In many cases, the phenotype has been directly linked to ΔFosB expression in NAc per se by use of viral-mediated gene transfer. In contrast to the behaviors listed in the table that are affected by ΔFosB, no effect has been seen on spatial learning in the Morris water maze.

ΔFosB homodimers form in vivo and what physiological function they subserve.

Drug induction of ΔFosB in the NAc seems to be a response to the pharmacological properties of the drug per se and not related to volitional drug intake, since animals that self-administer cocaine or receive yoked drug injections show equivalent induction of this transcription factor in this brain region [59]. As will be seen below, this is different from ΔFosB induction in certain other brain regions, which is dependent on volitional drug intake.

The 35–37 kD ΔFosB isoforms accumulate with chronic drug exposure due to their extraordinarily long half-lives [1, 8]. In contrast, there is no evidence that the splicing of ΔFosB or the stability of its mRNA is regulated by drug administration. As a result of its stability, therefore, the ΔFosB protein persists in neurons for at least several weeks after cessation of drug exposure. We now know that this stability is due to two factors (Figure 1): (1) the absence from ΔFosB of two degron domains, which are present at the C-terminus of full length FosB and all other Fos family proteins and target those proteins to rapid degradation, and (2) the phosphorylation of ΔFosB at its N-terminus by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and casein kinase 2 and perhaps by other protein kinases [7, 67,74,75]. The mechanism by which phosphorylation of ΔFosB at Ser27 increases its stability remains unknown. The stability of the ΔFosB isoforms provides a novel molecular mechanism by which drug-induced changes in gene expression can persist despite relatively long periods of drug withdrawal. We have, therefore, proposed that ΔFosB functions as a sustained "molecular switch" that helps initiate and then maintain an addicted state [40,50,51].

3 Role of ΔFosB in NAc in regulating behavioral responses to drugs of abuse

Insight into the role of ΔFosB in drug addiction has come largely from the study of bitransgenic mice in which ΔFosB can be induced selectively within the NAc and dorsal striatum of adult animals [9,29]. Importantly, these mice overexpress ΔFosB selectively in dynorphin/D1-type medium spiny neurons in NAc and dorsal striatum, where the drugs are believed to induce the protein. The behavioral phenotype of these ΔFosB-overexpressing mice, which in certain ways resembles animals after chronic drug exposure, is summarized in Table 2. The mice show augmented locomotor responses to cocaine after acute and chronic administration [29]. They also show enhanced sensitivity to the rewarding effects of cocaine and of morphine in place conditioning assays [29,87], and self-administer lower doses of cocaine than littermates that do not overexpress ΔFosB [13]. Likewise, such ΔFosB expression makes animals more sensitive to cocaine's ability to lower brain stimulation reward thresholds, further evidence of greater reward sensitivity [47]. Mice overexpressing ΔFosB also work harder to self-administer cocaine in progressive ratio self-administration assays, suggesting that ΔFosB increases the drive for cocaine and might thereby lead to a propensity for relapse during drug withdrawal [13]. As well, ΔFosB overexpression in NAc exaggerates the development of opiate physical dependence and promotes opiate analgesic tolerance [87]. In contrast, ΔFosB expressing mice are normal in several other behavioral domains, including spatial learning as assessed in the Morris water maze [29].

Specific targeting of ΔFosB overexpression to the NAc, by use of viral-mediated gene transfer, has yielded equivalent data [19,87], which indicates that this particular brain region can account for the phenotype observed in the bitransgenic mice, where ΔFosB is also expressed in dorsal striatum and to a lesser extent in certain other brain regions. Moreover, targeting enkephalin/D2-type medium spiny neurons in NAc and dorsal striatum in different lines of bitransgenic mice fail to show most of these behavioral phenotypes, which specifically implicates dynorphin/D1-type NAc neurons in these phenomena. In

contrast to overexpression of ΔFosB, overexpression of a truncated Jun protein (ΔcJun or ΔJunD)—which functions as a dominant negative antagonist of AP-1 mediated transcription—by use of bitransgenic mice or viral-mediated gene transfer, produces the opposite behavioral effects [56, 87]. Together, these data indicate that induction of ΔFosB in dynorphin/D1-type medium spiny neurons of the NAc is both necessary and sufficient to increase an animal's sensitivity to cocaine and other drugs of abuse, to mediate a relatively prolonged period of drug sensitization, and to promote drug intake and drug-seeking behavior, and support the view, stated above, that ΔFosB functions as a sustained molecular switch for the addicted state. An important question under current investigation is whether ΔFosB accumulation during drug exposure promotes drug-seeking behavior after extended withdrawal periods, even after ΔFosB levels have normalized (see below).

4 Induction of ΔFosB in NAc by natural rewards

The NAc is believed to function normally by regulating responses to natural rewards, such as food, drink, sex, and social interactions. As a result, there is a considerable interest in a possible role of this brain region in the so-called natural addictions (e.g., pathological over-eating, gambling, exercise, etc.). Animal models of such conditions are limited, nevertheless, we and others have found that high levels of consumption of several types of natural rewards lead to the accumulation of the stable 35–37 kD isoforms of ΔFosB in NAc. This has been seen after high levels of wheel running [81] as well as after chronic consumption of sucrose, high fat food, or sex [20, 61, 72, 79]. In some cases, this induction is selective for dynorphin/D1-type medium spiny neurons [81], but unknown for other cases. Moreover, studies of inducible, bitransgenic mice and of viral-mediated gene transfer have demonstrated that overexpression of ΔFosB in NAc increases the drive and consumption for each of these natural rewards, while overexpression of a dominant negative Jun protein exerts the opposite effect [20, 55, 61, 72, 73, 79, 81]. These findings suggest that ΔFosB in this brain region sensitizes animals not only for drug rewards, but for natural rewards as well, and thereby drives a higher motivational state for rewards in general and could possibly contribute to syndromes of natural addiction.

5 Induction of ΔFosB in NAc by chronic stress

Given the substantial evidence that ΔFosB is induced in NAc by chronic exposure to drug and natural rewards, it was interesting to observe that ΔFosB is also highly induced in this brain region after several forms of chronic stress, including restraint stress, unpredictable stress, and social defeat [58, 78]. Unlike drugs and natural rewards, however, this induction is seen more broadly in this brain region in that it is observed prominently in both dynorphin/D1- and enkephalin/D2-type medium spiny neurons.

This induction of ΔFosB represents a positive, coping response that helps an individual adapt to the stress, that is, ΔFosB promotes resilience. This hypothesis is supported by the observations that overexpression of ΔFosB in NAc, by use of inducible, bitransgenic mice or viral-mediated gene transfer, exerts antidepressant-like responses in several behavioral assays, while ΔcJun expression causes prodepression-like effects [78]. Moreover, the ability of chronic administration of standard antidepressants to alleviate the deleterious responses to chronic stress is blocked by ΔcJun or ΔJunD expression in the NAc. Interestingly, another form of chronic stress, prolonged social isolation of adult mice, leads to a reduction in basal levels of ΔFosB in the NAc and the prodepression-like effects of such prolonged isolation are blocked by ΔFosB overexpression in this brain region [78]. These findings are of particular interest because depressed humans display lower basal levels of ΔFosB in this brain region [78]. Together, these various lines of evidence are consistent with the view that ΔFosB in NAc increases motivational state and sensitivity towards natural rewards and may thereby help animals cope under periods of stress. This hypothesized role for ΔFosB in NAc is similar to that shown for periaqueductal gray and certain regions of prefrontal cortex, where the transcription factor is also induced by chronic stress and mediates resilience [4, 33].

6 Mechanism of ΔFosB induction in NAc

The upstream signaling pathways through which drugs, natural rewards, and stress induce ΔFosB in NAc remain largely unknown. Dopamine D1 receptor activation was shown to be required for ΔFosB induction in response to chronic cocaine [44, 53], consistent with its selective induction in D1-type NAc and dorsal striatal neurons, but little is known about mechanisms involved with the other stimuli. Recent work, however, has defined the transcription factors that are required for ΔFosB induction by cocaine and stress. The ability of cocaine to induce ΔFosB in NAc requires both CREB (cAMP response element binding protein) and SRF (serum response factor): local knockout of both transcription factors completely blocks cocaine's induction of ΔFosB, whereas knockout of either factor alone is without effect [77]. Surprisingly, only SRF is required for stress induction of ΔFosB in this same brain region: local knockout of SRF completely blocks the ability of stress to induce ΔFosB, whereas knockout of CREB is without effect [76]. These observations demonstrate that different stimuli can invoke different molecular mechanisms to induce ΔFosB even within the same brain region and presumably cell type. Further work is needed to understand the molecular basis of such stimulus-specific actions.

7 Target genes for ΔFosB in NAc

Since ΔFosB is a transcription factor, it presumably produces its interesting behavioral phenotypes in NAc by enhancing or repressing expression of other genes. As shown in Figure 1, ΔFosB is a truncated product of the *fosB* gene that lacks most of the C-terminal transactivation domain present in full-length FosB but retains the dimerization and DNA binding domains. ΔFosB dimerizes with Jun family members and binds AP-1 sites in DNA. Some in vitro studies suggest that, because ΔFosB lacks much of its transactivation domain, it functions as a negative regulator of AP-1 activity, while several others show that ΔFosB can activate transcription at AP-1 sites [8,15,48,85].

Using our inducible, bitransgenic mice that overexpress ΔFosB or its dominant negative ΔcJun, and analyzing gene expression on Affymetrix chips, we demonstrated that—in the NAc in vivo—ΔFosB functions primarily as a transcriptional activator, while it does serve as a repressor for a smaller subset of genes [39]. Interestingly, this differential activity of ΔFosB is a function of the duration and degree of ΔFosB expression, with short-term, lower levels leading to more gene repression, and long-term, higher levels leading to more gene activation. This is consistent with the finding that short-term and long-term ΔFosB expressions lead to opposite effects on behavior: short-term ΔFosB expression, like the expression of ΔcJun, reduces cocaine preference, while longer-term ΔFosB expression increases cocaine preference [39]. The mechanism responsible for this shift is currently under investigation; one novel possibility, which remains speculative, is that ΔFosB, at higher levels, may form homodimers that activate AP-1 transcription [25].

Several target genes of ΔFosB have been established using a candidate gene approach (Table 3). One target is GluA2 (GluR2), an AMPA glutamate receptor subunit [29]. ΔFosB overexpression in inducible bitransgenic mice selectively increases GluA2 expression in NAc, with no effect seen on several other AMPA glutamate receptor subunits analyzed, while ΔcJun expression blocks the ability of cocaine to upregulate GluA2 [56]. AP-1 complexes comprised of ΔFosB bind a consensus AP-1 site present in the GluA2 promoter. Furthermore, GluA2 overexpression via viral-mediated gene transfer increases the rewarding effects of cocaine, much like prolonged ΔFosB overexpression [29]. Since GluA2-containing AMPA channels are impermeable to Ca^{2+} and have a lower overall conductance compared to AMPA channels that do not contain this subunit, the cocaine- and ΔFosB-mediated upregulation of GluA2 in NAc could account, at least in part, for the reduced glutamatergic responses seen in these neurons after chronic drug exposure [19, 28]. Interestingly, more recent work has implicated ΔFosB induction of GluA2 in mediating the pro-resilience effects of this transcription factor in chronic stress models [78]. Such regulation of glutamatergic transmission is consistent with ΔFosB mediating the ability of cocaine to induce dendritic spines on dynorphin/D1-type medium spiny neurons [32, 65]. Indeed, recent evidence has shown that ΔFosB is both necessary and sufficient for this morphological adaptation to drug exposure, which is selective for D1-type neurons [19, 38]. ΔFosB also regulates electrophysiological responses of AMPA receptors in these neurons [19].

Table 3: Examples of validated target genes for ΔFosB in NAc[1].

Target	*Functional response*
↑ GluA2	Decreased sensitivity to glutamate, silent synapses
↓ Dynorphin	Downregulation of κ opioid feedback loop
↑ Cdk5	Expansion of dendritic processes
↓ NF-κB	Expansion of dendritic processes; regulation of cell survival pathways
↓ c-Fos	Molecular switch from short-lived Fos family proteins induced acutely to ΔFosB induced chronically

[1] ΔFosB regulates the expression of numerous genes in brain (e.g., [39,63]); only a few examples are shown. All of the genes included here meet at least four of the following criteria: (1) increased (↑) or decreased (↓) expression upon ΔFosB overexpression; (2) reciprocal or equivalent regulation by ΔcJun, a dominant negative inhibitor of AP-1-mediated transcription; (3) ΔFosB-containing AP-1 complexes bind to AP-1 sites in the promoter region of the gene; (4) ΔFosB causes a similar effect on gene promoter activity in vitro as seen in vivo; and (5) drug exposure induces increased binding of endogenous ΔFosB to the gene promoter.

Another candidate target gene of ΔFosB in NAc is the opioid peptide, dynorphin. Recall that ΔFosB appears to be induced by some drugs of abuse specifically in dynorphin/D1-type cells in this brain region. Drugs of abuse have complex effects on dynorphin expression, with increases or decreases seen depending on the treatment conditions used. The dynorphin gene contains AP-1-like sites, which can bind ΔFosB-containing AP-1 complexes. Moreover, we have shown that induction of ΔFosB represses dynorphin gene expression in NAc [87]. Dynorphin is thought to activate κ opioid receptors on VTA dopamine neurons and inhibit dopaminergic transmission and thereby downregulate reward mechanisms [70]. Hence, ΔFosB repression of dynorphin expression could contribute to the enhancement of reward mechanisms mediated by this transcription factor. There is now a direct evidence supporting the involvement of dynorphin gene repression in ΔFosB's behavioral phenotype [87].

Recent evidence has shown that ΔFosB also represses the *c-fos* gene which helps create the molecular switch—from induction of several short-lived Fos family proteins after acute drug exposure to the predominant accumulation of ΔFosB after chronic drug exposure—cited earlier [62]. The mechanism responsible for ΔFosB repression of *c-fos* expression is complex and is covered below.

The second approach used to identify target genes of ΔFosB in NAc is unbiased and utilizes genome-wide methods. First, we have identified genes whose expression levels are up- or downregulated upon the inducible overexpression of ΔFosB (or ΔcJun) in NAc using DNA expression arrays, as described earlier [2,10,12,39]. In parallel, we are using chromatin immunoprecipitation (ChIP) coupled with promoter arrays (ChIP-chip) or deep sequencing (ChIP-seq) to identify genes where the binding of endogenous ΔFosB is regulated by cocaine exposure [63] (see Section 9 on *Epigenetic Mechanisms*). This unbiased approach has led to the identification of a large number of putative target genes for ΔFosB in NAc. Two genes which appear to be induced through ΔFosB's actions as a transcriptional activator are cyclin-dependent kinase-5 (CDK5) and its cofactor P35 [5,39]. CDK5 is also induced by chronic cocaine in the NAc, an effect blocked upon ΔcJun expression, and ΔFosB binds to and activates the CDK5 gene through an AP-1 site in its promoter [12,56]. CDK5 is an important target of ΔFosB since its expression has been directly linked to changes in the phosphorylation state of numerous synaptic proteins including glutamate receptor subunits [5], as well as increases in dendritic spine density [52], in the NAc that are associated with chronic cocaine administration. Regulation of CDK5 activity in NAc has also been linked directly to alterations in the behavioral effects of cocaine [71].

Another ΔFosB target identified by use of microarrays is NFkB. This transcription factor is induced in NAc by ΔFosB overexpression and by chronic cocaine, an effect blocked by ΔcJun expression [2,56]. Recent evidence suggests that induction of NFkB may also contribute to cocaine's and ΔFosB's ability to induce dendritic spines and mediate sensitized behavioral responses in NAc neurons [68]. As well, NFkB has been implicated in some of the neurotoxic effects of methamphetamine in striatal regions [3]. The observation that NFkB is a target gene for ΔFosB emphasizes the complexity of the mechanisms by which ΔFosB mediates the effects of cocaine on gene expression. Thus, in addition to genes regulated by ΔFosB directly via AP-1 sites on the gene promoters, ΔFosB would be expected to regulate many additional genes via altered expression of NFkB and presumably other transcriptional regulatory proteins.

Still another ΔFosB target identified by our open-ended approach is the sirtuins (SIRTs), protein deacetylases that regulate gene expression in the nucleus as well as several other cellular processes via cytoplasmic actions [63]. SIRT1 and SIRT2 are induced in NAc by chronic cocaine administration and this induction is mediated via ΔFosB. Such induction seems to contribute to ΔFosB's behavioral effects, since SIRT activation in NAc promotes behavioral responses to cocaine, whereas blockade of SIRT has the opposite effects [17,63].

Genome-wide gene and chromatin assays, which are ongoing, are providing a rich list of many additional genes that may be targeted—directly or indirectly—by ΔFosB. Among these genes are additional neurotransmitter receptors, proteins involved in pre- and postsynaptic function, many types of ion channels and intracellular signaling proteins, as well as proteins that regulate the neuronal cytoskeleton and cell growth [39,63]. Further work is needed to confirm each of these numerous proteins as bona fide targets of cocaine acting through ΔFosB and to establish the precise role that each protein plays in mediating the complex neural and behavioral aspects of cocaine action. Ultimately, of course, it will be crucial to move beyond the analysis of individual target genes to the regulation of groups of genes whose coordinated regulation is likely required to mediate the addicted state.

8 Induction of ΔFosB in other brain regions

The discussion up to now has focused solely on NAc. While this is a key brain reward region and important for the addicting actions of cocaine and other drugs of abuse, many other brain regions are also crucial in the development and maintenance of a state of addiction. An important question, then, is whether ΔFosB acting in other brain regions beyond the NAc may also influence drug addiction. Indeed, there is now increasing evidence that stimulant and opiate drugs of abuse induce ΔFosB in several brain regions implicated in several aspects of addiction [27,37,41,42,53,54,57,59].

One study systematically compared ΔFosB induction in these various brain regions across four different drugs of abuse: cocaine, morphine, cannabinoids, and ethanol [59]. All four drugs induce the transcription factor to varying degrees in NAc and dorsal striatum as well as in prefrontal cortex, amygdala, hippocampus, bed nucleus of the stria terminalis, and interstitial nucleus of the posterior limb of the anterior commissure. Cocaine and ethanol alone induce ΔFosB in lateral septum, all of the drugs except for cannabinoids induce ΔFosB in the periaqueductal grey, and cocaine is unique in inducing ΔFosB in GABAergic cells in the posterior ventral tegmental area [57,59]. In addition, morphine has been shown to induce ΔFosB in ventral pallidum and several regions of prefrontal cortex, among other regions [27,41]. In each of these brain areas, it is the 35–37 kD isoforms of ΔFosB that accumulate with chronic drug exposure and persist through relatively long periods of withdrawal.

A major goal for future research is to carry out studies, analogous to those described above for NAc, to delineate the neural and behavioral phenotype mediated by ΔFosB for each of these brain regions. This represents an enormous undertaking, yet it is crucial for understanding the global influence of ΔFosB on the addiction process.

We have taken a significant step in this regard by using viral-mediated gene transfer to characterize the actions of ΔFosB in a particular subregion of prefrontal cortex, namely, orbitofrontal cortex. This region has been strongly implicated in addiction, in particular, in contributing to the impulsivity and compulsivity that characterizes an addicted state [26]. Interestingly, unlike the NAc where self-administered and yoked cocaine induce comparable levels of ΔFosB as noted earlier, we observed that cocaine self-administration causes a several-fold greater induction of ΔFosB in orbitofrontal cortex, suggesting that this response may be related to volitional aspects of drug administration [84]. We then used rodent tests of attention and decision-making (e.g., 5 choice serial reaction time and delay discounting tests) to determine whether ΔFosB within the orbitofrontal cortex contributes to drug-induced alterations in cognition. We found that chronic cocaine treatment produces tolerance to the cognitive impairments caused by acute cocaine. Viral-mediated overexpression of ΔFosB within this region mimicked the effects of chronic cocaine, while overexpression of the dominant negative antagonist, ΔJunD, prevents this behavioral adaptation. Induction of ΔFosB in orbitofrontal cortex also promotes sensitization to the locomotor-activating effects of the drug as well as the impulsivity that occurs during withdrawal from cocaine self-administration [82,83]. DNA expression microarray analyses identified several potential molecular mechanisms underlying this behavioral change, including a cocaine- and ΔFosB-mediated increase in the transcription of the metabotropic glutamate receptor mGluR5 and $GABA_A$ receptor subunits, as well as substance P [84]. The influence of these and many other putative ΔFosB targets requires further investigation.

These findings indicate that ΔFosB helps mediate tolerance to the cognitive-disrupting effects of cocaine. Users who experience tolerance to the deleterious effects of cocaine are more likely to become cocaine-dependent, whereas those who find the drug more disruptive at work or school are less likely to become addicted [69]. Tolerance to the cognitive disruption caused by acute cocaine in cocaine-experienced individuals, as well as enhanced impulsivity, may thus facilitate the maintenance of addiction. In this way, ΔFosB induction in the orbitofrontal cortex may promote an addicted state, similar to its actions in the NAc where ΔFosB promotes addiction by enhancing the rewarding and incentive motivational effects of the drug.

9 Epigenetic mechanisms of ΔFosB action

Until recently, all studies of transcriptional regulation in brain have relied on measurements of steady state mRNA levels. For example, the search for ΔFosB target genes has involved identifying mRNAs up- or downregulated upon ΔFosB or ΔcJun overexpression, as stated earlier. This level of analysis has been very useful in identifying putative targets for ΔFosB, however, it is inherently limited in providing insight into the underlying mechanisms involved. Thus, all studies of mechanisms have relied on in vitro measures such as ΔFosB binding to a gene's promoter sequences in gel shift assays or ΔFosB regulation of a gene's promoter activity in cell culture. This is unsatisfying because mechanisms of transcription regulation show dramatic variations from cell type to cell type, leaving it virtually completely unknown how a drug of abuse, or ΔFosB, regulate their specific genes in the brain in vivo.

Studies of epigenetic mechanisms make it possible, for the first time, to push the envelop one step further and directly examine transcriptional regulation in the brains of behaving animals [66]. Historically, the term epigenetics has been used to describe mechanisms by which cellular traits can be inherited without a change in DNA sequence. We use the term more broadly to encompass "the structural adaptation of chromosomal regions so as to register signal or perpetuate altered activity states" [6]. Thus, we now know that the activity of genes is controlled by the covalent modification (e.g., acetylation and methylation) of histones in the genes' vicinity and the recruitment of diverse types of coactivators or corepressors of transcription. ChIP assays make it possible to take advantage of this growing knowledge of chromatin biology to determine the activation state of a gene in a particular brain region of an animal treated with a drug of abuse, along with the detailed constellation of proteins that control that state of activation.

Examples of how studies of chromatin regulation can help us understand the detailed molecular mechanisms of action of cocaine and ΔFosB are given in Figure 3. As stated above, ΔFosB can function as either a transcriptional activator or repressor depending on the target gene involved. To gain insight into these actions, we analyzed the chromatin state of two representative gene targets for ΔFosB, *Cdk5* which is induced by ΔFosB and *c-Fos* which is repressed, in NAc. ChIP studies demonstrate that cocaine activates the *Cdk5* gene in this brain region through the following cascade: ΔFosB binds to the gene and then recruits histone acetyltransferases (which acetylate nearby histones) and SWI-SNF (SWItch/Sucrose NonFermentable) factors; both actions promote gene transcription [6,30,35]. Chronic cocaine further augments histone acetylation through the phosphorylation and inhibition of histone deacetylases (which normally deactylate and repress genes) [34,64]. In contrast, cocaine represses the *c-Fos* gene: when ΔFosB binds to this gene it recruits histone deacetylases and histone methyltransferases (which methylate nearby histones) and thereby inhibits *c-Fos* transcription (Figure 3) [62]. A central question is: what determines whether ΔFosB activates or represses a gene when it binds to that gene's promoter? We hope to get at this question with our genome-wide ChIP-seq

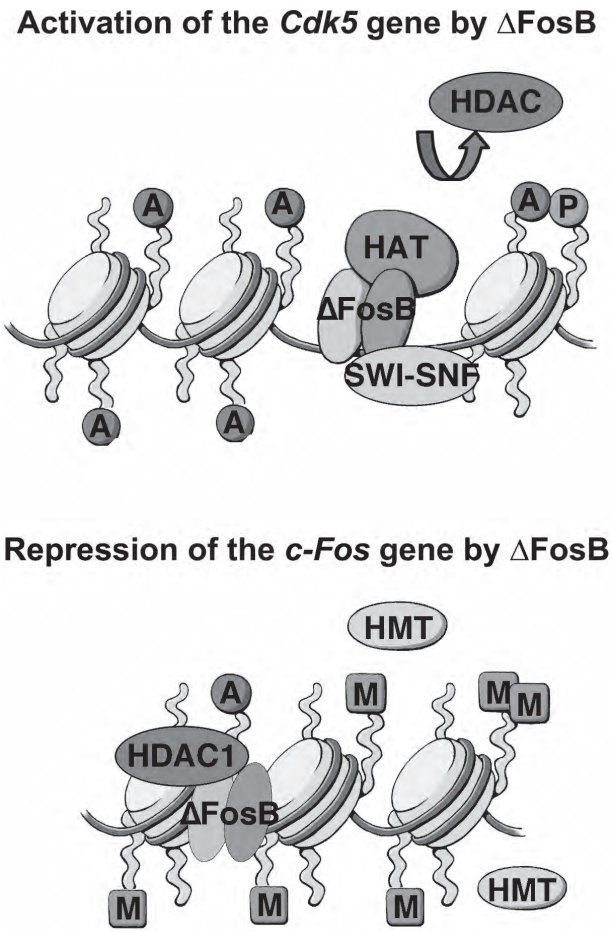

Figure 3: *Epigenetic mechanisms involved in ΔFosB action.* The figure illustrates the very different consequences when ΔFosB binds to a gene that it activates (e.g., *Cdk5*) versus represses (e.g., *c-Fos*). At the *Cdk5* promoter [*Top*], ΔFosB recruits histone acetyltransferases (HAT) and SWI-SNF factors, which promote gene activation. There is also evidence for exclusion of histone deacetylases (HDAC) (see text). In contrast, at the *c-Fos* promoter [*Bottom*], ΔFosB recruits HDACs as well as histone methytransferases (HMT), which repress gene expression. A, P, and M depict histone acetylation, phosphorylation, and methylation, respectively.

experiments, which will tell us whether ΔFosB's effect on a given gene is encoded in the primary sequence around the ΔFosB binding site or, rather, encoded in the general chromatin landscape of that gene.

Beyond controlling steady-state mRNA levels, epigenetic regulation also controls the inducibility of a gene in response to some subsequent stimulus. For example, we have shown that drug-induced epigenetic modifications are important in priming or desensitizing certain genes for greater or reduced regulation in response to re-exposure to the drug even after long withdrawal periods [31, 38, 66]. Interestingly, the *FosB* gene exhibits this type of latent epigenetic regulation. Thus, we have demonstrated enhanced induction of ΔFosB mRNA and protein in NAc, but not dorsal striatum, after one month of withdrawal from chronic cocaine administration, and such enhanced induction is associated with epigenetic modifications that would be expected to prime the gene for greater inducibility [14]. In contrast, no changes were observed in the signaling pathways upstream of *FosB* gene regulation. Such epigenetic priming could provide one mechanism by which behavioral abnormalities associated with addiction arise much more rapidly in drug-experienced individuals compared to those without a history of drug exposure.

These early studies of epigenetic mechanisms of drug addiction are exciting because they promise to reveal fundamentally new information concerning the molecular mechanisms by which drugs of abuse regulate gene expression in NAc and other brain regions. Epigenetic mechanisms are particularly attractive candidates to mediate the very long-lived phenomena central to a state of addiction. In this way, drug- and ΔFosB-induced changes in histone modifications and related epigenetic alterations provide potential mechanisms by which transcriptional changes can persist long after drug exposure ceases and perhaps even after ΔFosB degrades to normal levels.

10 Conclusions

The pattern of induction of ΔFosB in NAc by chronic exposure to natural rewards, stress, or drugs of abuse raises an interesting hypothesis concerning the protein's normal functioning in this brain region. As depicted in Figure 2, there is an appreciable level of ΔFosB in NAc under normal conditions. This is unique to striatal regions, as ΔFosB is virtually undetectable elsewhere throughout brain at baseline. We hypothesize that levels of ΔFosB in NAc represent a readout of an individual's exposure to emotional stimuli, both positive and negative, integrated over relatively long periods of time given the temporal properties of the protein. The partial differences in the cellular specificity of ΔFosB induction by rewarding versus aversive stimuli (i.e., induction in D1-type vs. D2-type medium spiny neurons) are poorly understood, and further work is needed to elucidate the functional consequences of these distinctions. We hypothesize further that as higher levels of emotional stimulation induce more ΔFosB in NAc neurons, the neurons' functioning is altered so that they become more sensitive to rewarding stimuli. In this way, induction of ΔFosB would promote reward-related (i.e., emotional) memory through afferent projections of the NAc. Under normal circumstances, induction of moderate levels of ΔFosB by rewarding or aversive stimuli would be adaptive by enhancing an animal's adjustments to environmental challenges. However, the excessive induction of ΔFosB seen under pathological conditions (e.g., chronic

exposure to a drug of abuse) would lead to excessive sensitization of the NAc circuitry and ultimately contribute to pathological behaviors (e.g., compulsive drug seeking and taking) associated with drug addiction. ΔFosB induction in other brain regions would presumably contribute to distinct aspects of an addicted state, as suggested by ΔFosB action in orbitofrontal cortex.

If this hypothesis is correct, it raises the interesting possibility that levels of ΔFosB in NAc or perhaps other brain regions could be used as a biomarker to assess the state of activation of an individual's reward circuitry, as well as the degree to which an individual is "addicted," both during the development of an addiction and its gradual waning during extended withdrawal or treatment. The use of ΔFosB as a marker of a state of addiction has been demonstrated in animal models. Adolescent animals show much greater induction of ΔFosB compared to older animals, consistent with their greater vulnerability for addiction [16]. As well, attenuation of the rewarding effects of nicotine or alcohol with pharmacological or electrical stimulation approaches is associated with reductions in ΔFosB induction in NAc [36, 43]. Although highly speculative, it is conceivable that a small molecule PET ligand, with high affinity for ΔFosB, could be used to help diagnosis addictive disorders as well as monitor progress during treatment.

Finally, ΔFosB itself, or any of the numerous genes it regulates—identified through DNA expression arrays or ChIP on chip assays—represents potential targets for the development of fundamentally novel treatments for drug addiction. As just one example, we are in the process of screening for small molecules that enhance or inhibit the actions of ΔFosB [80]. We believe it is imperative to look beyond traditional drug targets (e.g., neurotransmitter receptors and transporters) for potential treatment agents for addiction. The genome-wide transcriptional maps capable with today's advanced technologies provide a promising source of such novel targets in our efforts to better treat and ultimately cure addictive disorders.

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