Review

Transcription of Fos family genes in nucleus accumbens: roles of AP-1, epigenetics, and stochasticity

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We elaborate upon further details of the mechanisms involved in transcription of Fos family and other immediate early genes in brain. The AP-1 element on the c*fos* promoter is bound by homodimers of Jun family proteins and heterodimers of Jun family members with Fos family members including ΔFosB. The frequencies of combinations are discussed, as well as their activities, which may be activating or inhibiting. With acute stimulation by psychostimulants the mRNAs of c*fos* and *fos*B are induced within a few minutes to rise to maximum levels in about 30 minutes with a decline to basal levels in a few hours. The many possible mechanisms for the shutdown are discussed. Epigenetic modifications are strongly implicated in the instigation and inhibition of transcription, particularly histone modifications which may decompactify DNA. Summaries of histone modifications have been related to a histone code. Various general schemes for the steps to transcription have been proposed and three of these are described, followed by more detailed dynamical pictures. Finally, results for transcription measurements in single cells are discussed and some simple mathematical models that have been employed to quantify their stochastic properties are described.

Keywords: transcription, ΔFosB, IEG, AP-1, epigenetic mechanisms, psychostimulants, stochastic bursting

1. Introduction: Role of AP-1 complexes

An AP-1 (activator protein-1) complex is a dimer of protein molecules, such as members of Fos or Jun families, bound together with a leucine zipper (Landschulz et al., 1988). **Figure 1** illustrates such a complex bound to DNA.

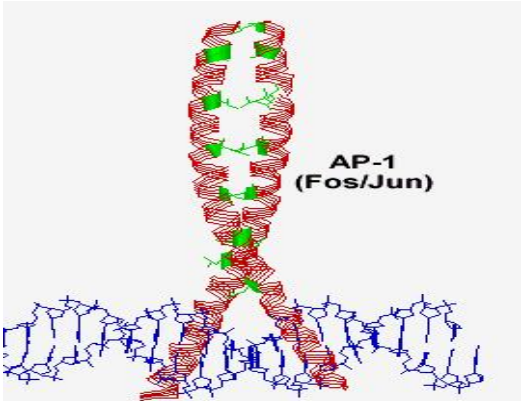

Figure 1 An AP-1 complex of a Fos and a Jun protein showing the molecular structure of the leucine zipper binding the two proteins.

Such AP-1 complexes act as transcription factors by binding to special AP-1 sites on DNA within promoters. Jun proteins can form both homo and heterodimers, but Fos proteins only form AP-1 pairs with a Jun family protein. The Jun-Fos heterodimer is generally more stable, with greater DNA-binding activity than Jun homodimers. All Fos family members, including Δ*fos*B, participate in forming AP-1 pairs. It has been stated that cFos and other fos proteins only activate genes, that is act as transcription factors, if they are part of an AP-1 pair (Yen et al., 1991; Benito & Barco, 2015; Ohnishi et al., 2015; Barger, 2016) which must involve a Jun family protein. Since cFos, for example, has often been claimed to be autorepressive (König et al., 1989; Morgan & Curran, 1991; Angel & Karin, 1991; Mumberg et al., 1991), and a possible mechanism is via cFos itself, it is important to include Jun family proteins in modeling the dynamics of cFos induction.

Considering the AP-1 complexes formed by the three Jun family proteins cJun, JunB, JunD with the five Fos family proteins cFos, FosB, Fra1, Fra2, ΔFosB (not distinguishing the three forms with molecular masses 33, 35 and 37 kDa) there are are 21 different dimers of which 6 are homodimers (jun/jun) and 15 heterodimers (fos/jun). Interactions between various Fos-Jun family members and more than 50 different proteins have been reported (**Table 1** of Chinenov & Kerppola, 2001). For example, several members of the ATF group of proteins form heterodimers with Fos and Jun family proteins. Such pairs preferentially interact with CRE rather than AP-1 sites.

1.1 Frequencies of combinations

In vitro Fos-Jun complexes may form extremely rapidly (within a few minutes) and are very stable compared to Jun-Jun pairs (Rauscher et al., 1988). ΔFosB and other Fos family proteins can form active AP-1 heterodimers with any of the Jun family proteins (Suzuki et al., 1992; Chen et al., 1995; Hughes and Dragunow, 1995; McClung et al., 2004; Ohnishi et al., 2015). With prolonged stimulation by cocaine, ΔFosB forms long-lasting AP-1 heterodimers which may underly neuronal plasticity associated with addiction (Nestler, 2004).

AP-1 binding parallels the strength of the induction of Fos family and Jun family proteins. The amount of a specific heterodimer formed between distinct Fos and Jun proteins at a given time depends, essentially, on the relative amounts of each protein present at that time (Kovary & Bravo, 1991, 1993).

The complexity of the response to stimulation leading to the transcription of many IEGs was highlighted by Nakabeppu & Nathans (1991), based on in vitro studies, who noted that c*jun*, *jun*B, *jun*D, c*fos*, *fra*1, *fra*2 and FosB are all induced, and this can lead to the formation of numerous dimers, each one having a different effect on transcription. These various combinations must compete at AP-1 sites.

A study of Kovary & Bravo (1993) found that Fos-Jun heterodimers form rapidly. In Swiss 3T3 cells at 1 hour JunB is the major partner of cFos because JunB is the most abundant of the Jun proteins. cFos was more stable when combined with a Jun protein. Later, heterodimers of JunB with Fra1 and Fra2 appear.

In brain, JunD mRNA is constitutively expressed in cerebral cortex and striatum and is not affected by chronic ECS in the former or chronic cocaine in the latter. Thus, in such chronic experiments, the most frequent and long-lasting AP-1 heterodimers are with ΔFosB and JunD and to a lesser extent JunB (Chen et al., 1995; Nestler, 2015a).

1.2 Effects of combinations

The activity of an AP-1 complex depends on the cell type (Chinenov & Kerppola, 2001), and on the phosphorylation state of each partner (Hipskind & Bilbe, 1998). Barger (2016) cited a study which found that the contribution of cFos to AP-1 activity depends primarily on its quantity, whereas the state of phosphorylation of Jun partners is a key factor in determining their contribution.

The heterodimeric AP-1 pairs formed by Fos family and Jun family members may have either an enhancing (e.g., Sonnenberg et al., 1989) or inhibiting (Chen et al. , 1993) effect on transcription (Ohnishi et al., 2015). Suzuki et al. (1992) found that Fra2/cJun was suppressive whereas Fra2/JunD was activating. With the application of chronic ECS or chronic cocaine, the heterodimers formed by JunD and ΔFosB are likely to have a negative effect on AP-1 mediated transcription in cerebral cortex and striatum, repectively (Chen et al., 1995). However, ΔFosB-jun heterodimers can have positive or negative effects on the expression of various genes in the NAc (Nestler, 2008).

Hughes & Dragunow (1995) compiled a table listing the relative strengths of various AP-1 pairs, according to which cFos or FosB combined with either cJun or JunD have high transactivational activity, whereas cFos or FosB combined with JunB are of medium activity as are Fra1 or Fra2 combined with JunD. Significant but weak activity occurs with cJun paired with cJun and with JunD combined with either JunD or cJun. Nine other pairs were ascribed weak or negligible activity. However, these data were based on experiments with cultured cells transfected with an AP-1 containing promoter. Such results may not generalize to many mammalian cells.

As seen above, acute administration of most addictive drugs transiently increases the expression of several Fos and Jun family members with an increase in AP-1 binding activity in the NAc and dorsal striatum (McClung et al., 2004). Chronic drug application leads to the accumulation of ΔFosB due to the unusually large stability of some of its isforms. This leads to prolonged AP-1 binding activity and the activation of transcription which may persist for weeks after the cessation of drug treatment (Chen et al., 1997;

Dobrzanski et al., 1991; Chen et al., 2000; McClung et al., 2003). As summarized in Nestler (2008) in striatum and NAc, the long-lasting forms of ΔFosB form active AP-1 dimers mainly with JunD and to a lesser extent JunB (Chen et al., 2000). Some reports indicated that these dimers negatively regulated AP-1 activity, whereas others found they activated transcription. A recent study showed that AP-1 pairs of cFos and JunB were involved in spinal cord regeneration after injury in salamander; in contrast cFos, cJun AP-1 dimers played a role in recovery after mammalian CNS injury (Sabin et al., 2019).

2. Repression and transrepression of IEG transcription

As seen above, many IEGs are activated by various stimuli in a variety of types of cell, including neurons. Usually observed in response to acute stimulation are mRNA levels increasing within minutes to rapidly reach a maximum after about 30 minutes and then declining to basal values within a few hours. According to Cochran (1995) the number of copies of c*fos* can be in the range 600 to 1000 per cell but the numbers of mRNA involved are discussed further in section 5 of this article.

The mechanisms for the shut-down of transcription have been investigated since the late 1980s, but there has been expressed uncertainty about the processes involved (Nestler, 2017, *personal communication*). To describe accurately the dynamics of mRNA and protein production it is necessary to have an accurate theory of the mechanisms responsible for the shutdown of transcription of such genes as c*fos*, *fos*B and other IEG.

In what follows we will focus mainly on c*fos* which is the IEG most investigated and discussed. Some of the several factors which could contribute to the shutdown of transcription are as follows:

(i) cFos autorepression or transrepression by other Fos proteins such as FosB, Fra1, Fra2 due to interference with transcription at one or more elements, particularly the SRE, of the c*fos* promoter. This was conjectured by many to involve AP-1 binding but such a mechanism was not supported in many cases by the findings of others.

(ii) Decline of activity in signaling pathways coupled with rapid degradation of transcription factors.

(iii) Dephosphorylation of transcription factors. For example, if CREB is dephosphorylated at Ser133, it cannot activate transcription at the CRE.

(iv) Histone modifications such as deacetylation through an HDAC such as SIRT1 or methylation through an HMT such as G9a, involving a dimer of ΔFosB and constitutional JunD. The roles of histone modifications in the activation and shutoff of transcription are discussed in the following section on Epigenetic mechanisms.

2.1 *Discussion of these factors*

2.1.1 Autorepression and transrepression

The terms autorepression and transrepression refer to the negative effects of a protein on its own production or on the production of other proteins. That inhibitors of protein synthesis could lead to a much-reduced shutoff of transcription in some cases was an important development (Greenberg et al., 1986a; Mitchell, 1986; Sassone-Corsi et al, 1988). De novo proteins were thus implicated in the shutoff. Further analysis of such so called superinduction of c*fos* implicated cFos itself in the shutdown of c*fos* transcription, which led to the concept of autorepression (Ofir et al., 1990; Morgan & Curran, 1991; Dobryzanski et al., 1991; Cochran, 1995). However in some cases the opposite effect occurs. Whereas cFos and cJun negatively impact c*fos* transcription, the c*jun* gene is positively autoregulated by its product cJun (Angel et al., 1988), an effect probably mediated by cJun homodimers at AP-1 sites (Angel, 1991; Hughes & Dragunow, 1995). Of interest is the finding that Fra1 could inhibit AP-1 activity in some cases (Yoshioka et al., 1995).

Induction of *fos*B mRNA and production of FosB protein display similar, but somewhat slower, kinetics to c*fos*, which is related to their having very similar promoter elements (Lazo et al., 1992). These authors found that FosB and cFos can inhibit the activity of the *fos*B promoter to a similar extent, and furthermore. that the activity of the c*fos* SRE is downregulated by FosB and cFos so that both proteins have the capacity to transrepress the transcription of each other.

Furthermore, *fra*1 and *fra*2 have delayed responses relative to *fos*B and, as noted by Morgan & Curran (1991), Fra1 can cause repression of SRE mediated transcription at the c*fos* promoter (Gius et al., 1990). Sonnenberg et al. (1989) and Morgan & Curran (1991)

suggested that this could explain the long duration of the refractory period after c*fos* induction, which lasts many hours after cFos protein has disappeared as Fra1 and other fos family proteins (fras) are expressed throughout this time period. In fact Sonnenberg et al (1989) stated that in brain the phase of c*fos* repression correlates with expression of Fra but not cFos.

Mathematical modeling of these effects could interestingly and easily be considered as negative feedback of proteins on the corresponding transcription processes. It turns out, however, that such an attractively simple approach may not accurately describe the underlying biochemical processes.

Investigations of the mechanisms of repression involving cFos mostly concentrated on the role of the SRE element of the c*fos* promoter. From such investigations, differing views emerged. It was conjectured that the autorepression and transrepression of c*fos* and *fos*B transcription might involve the formation of AP-1 complexes involving Fos family and Jun family partners (Sassone-Corsi et al., 1988b; Robertson et al., 1995; Hughes & Dragunow, 1995; Herdegen & Leah, 1998; Kovács, 1998; Sng et al., 2004; Alibhai et al., 2007; Calais, 2013). Hughes and Dragunow (1995) stated that two regions of the c*fos* promoter seemed required for autorepression, including one encompassing the SRE and AP-1 elements. However, analysis by König et al. (1989), Lucibello et al. (1989) and Gius et al. (1990) indicated that the binding of dimers of Fos and Jun to an AP-1 site was not required for the autorepression of c*fos*. Angel (1991) claimed in fact that the AP-1 site of the c*fos* promoter was not involved in the mediation of c*fos* autorepression. However, Renthal et al. (2008) found that the repression or weakness in expression of c*fos* by chronic amphetamine was likely to be due to the recruitment by ΔFosB of an HDAC to form a dimer at an AP-1 site in the c*fos* promoter. HDAC is a mediator of deacetylation of histones, which usually negatively impacts on transcription.

The fact that some protein synthesis inhibitors led to sustained c*fos* transcription played a major role in the devleopment of the idea that cFos was autorepressing c*fos* transcription. However, Mahadevan et al. (1990) reported that protein synthesis inhibitors led to rapid phosphorylation, which was instrumental in promoting transcription. Thus, it seems that the superinduction phenomenon was probably not due to the suppression of shutoff by protein products but rather due to gene activation by histone modifications, as discussed in the following section. The experiments of Edwards & Mahadevan (1992) and Zinck et al. (1995) established that there was no labile repressor of c*fos* expression but that modifications of signaling pathways and transcription factors induced by some protein synthesis inhibitors were responsible for the phenomenon of superinduction.

2.1.2 Signaling decline and degradation

Sometimes, the rapid degradation and instability of cfos mRNA and/or protein is mentioned as contributing to the transient nature of c*fos* induction. It is certainly the case that newly transcribed mRNA and newly translated protein are often rapidly degraded, but these processes must follow transcription and are not a factor in the shutoff of transcription. Hope (1998) remarked that the downregulation of IEGs must involve either downregulation of an activating pathway such as through the SRE or SIE, or the up regulation of a repressing pathway. Furthermore, some potent transcription factors are quickly degraded (Carle et al., 2007), which could contribute to the rapid termination of c*fos* transcription.

2.1.3 Dephosphorylation of transcription factors

As remarked by Kovács (1998), dephosphorylation of transcription factors plays a significant role in the regulation of c*fos* expression, and it is possible that such processes are involved in the shut off of transcription of IEGs. The most studied mediators of dephosphorylation are the phosphatases PP1 and PP2A. One route to pCREB and subsequent transcription is through cAMP-dependent PKA as shown in **Figure 2**, though the details of the mechanisms involved may be different, as discussed fully in a subsequent article. Bito et al. (1996); Shaywitz & Greenberg (1999) and Mayr & Montminy (2001) contain comprehensive descriptions of the reactions which were posited to be involved. The latter reference lists 12 signals that lead to CREB phosporylation, including cAMP through PKA and Ca^{2+} through CaMKII, IV. Transient pCREB does not lead to c*fos* transcription which requires sustained Ser133 phosphorylation of CREB.

Mayr & Montminy (2001) presented convincing evidence that the phosphorylation and dephosphorylation processes, particularly those of CREB involving cAMP, correlated with transcription rate as illustrated in Figure 2. Here the rising phase of transcription lasting about 30 minutes corresponds to the phosphorylation of CREB by the catalytic subunits of PKA after entry to the nucleus and subsequent recruiting of the histone acetyltransferase CBP, which was known to expedite transcription. After the peak, dephosphorylation of CREB by PP1 and PP2A causes a decline in transcription. A recovery period follows in which the system is in a refractory state, attributable to the downregulation of the expression of the catalytic PKA subunits and the induction of repressors such as ICER (Shaywitz & Greenberg, 1999).

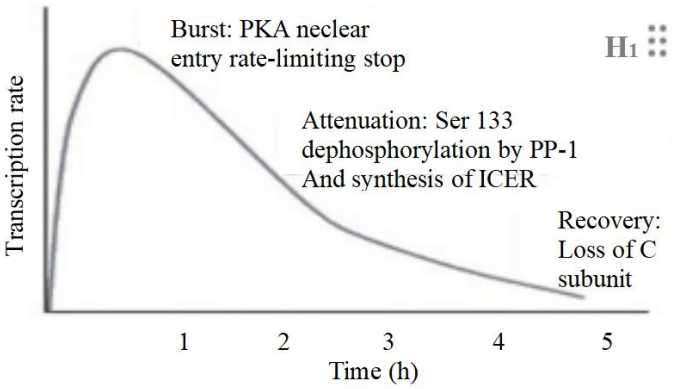

Figure 2 Showing how the time dependence of transcription parallels the phosphorylation and dephosphorylation of the transcription factor CREB. Adapted from Mayr & Montminy (2001).

3. Epigenetic mechanisms

Epigenetic mechanisms are defined as those which alter the properties of DNA without changing the DNA sequence. Within the nuclei of eukaryotic cells, DNA is compacted by wrapping around groups of eight core histone proteins, to form units called nucleosomes which are linked by linker DNA and linker histones (H1 or an isoform H5). Only genes occurring in spaced out nucleosomes tend to be transcribed, otherwise they are not accessible (Nestler, 2016). The main mechanisms responsible for such alterations in transcriptional access are histone modifications and DNA methylation (Kouzarides, 2007). A table of some known epigenetic modifications in response to cocaine, alcohol and opiods was given by Nielsen et al. (2012). Recently, various forms of noncoding RNAs, including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) have been studied for their roles in epigenetic regulation (Nestler, 2016).

Histones were posited as playing a role in gene activity many years ago (Stedman & Stedman, 1950), an hypothesis which was strongly supported by the work of Huang & Bonner (1962). An interesting account of some other details of the history of histone discovery and research is given by Barger (2016). The complexity of the nervous system, the signaling pathways and the various epigenetic mechanisms they induce have led to the description of such contributions to cognitive and behavioral phenomena as stochastic processes of a formidable dimensionality.

3.1 *Histone modifications*

There are two copies each of the core histones H2A, H2B, H3 and H4. The DNA, together with the histone complexes, is called chromatin. To facilitate transcription, the coils of DNA must be unraveled, a process along with condensation called chromatin remodeling in which histone modifications play a key role. Important such modifications, effected via signaling pathways, occur at the projecting core histone N-terminal tails consisting of amino acid strings. These are of various lengths from 16 to over 40 residues. H2A also has a C-terminal tail (Nestler, 2016). Histone dynamics, especially of variants such as H3.3, has added an extra dimension to the regulation and time course of transcription (Commerford et al., 1982; Ahmad & Henikoff, 2002; Neméth & Längst, 2004; Maze et al., 2015). The histone modifications include acetylation (add acetyl group, CH_3CO), phosphorylation (add phosphate group, PO_4), methylation (add methyl group, CH_3), ubiquitination (add the small regulatory ubiquitin protein) and polyADP-ribosylation (transfer of multiple ADP-ribose molecules) (Strahl & Allis, 2000; Nestler, 2014, 2016). Such processes may involve the recruitment of transcription coactivators or corepressors.

Generally, acetylation tends to promote transcription, whereas methylation can promote or inhibit transcription according to which histone tail amino acid is affected. Phosphorylation may also have positive or negative effects. However, a genome-wide study of the effects of chronic cocaine in the NAc (Renthal et al., 2009; Nestler, 2014) found that whereas many gene promoters did show a correlation between increased and decreased mRNA expression with hyperacetylation and hypoacetylation respectively, most genes did not.

Enzymes which catalyze acetylation of histones are called histone acetlyltransferases (HATs), those that reverse this process are called histone deacetylases (HDACs); those that mediate methylation are called histone methyltransferases (HMTs) and those that reverse this are called histone demethylases (HDMs). The most frequently studied such enzymes are the HAT, CREB-binding protein (CBP), and the HMTs, euchromatic histone-lysine N-methyltransferase (Ehmt2), or G9a, and GLP (G9a-like protein).

3.1.1 CBP

The induction of c*fos* and *fos*B in the striatum after acute cocaine is associated with transient increases in the acetylation of H4, but not of H3. This acetylation is mediated by CBP (Kumar et al., 2005; Levine et al., 2005; Brami-Cherrier et al. 2005) and is also found with administration of amphetamine (Renthal et al., 2008). It has been found that CBP involvement is critical for such transcription (Malvaez et al., 2011). Rogge & Wood (2013) noted that CREB is activated by phosphorylation at Ser133 and that such phosphorylation leads to the recruitment of CBP.

In the case of c*fos*, there is also H3 phosphoacetylation, these changes occurring within 30 minutes (see Kumar et al., 2005, for graphical results) and being evident for up to 3 hours. These authors conjectured that as phosphoacetylation of H3 is perhaps relatively specific for the c*fos* gene, it may contribute to the rapidity of its induction. The control of H3 phosphorylation by cocaine is mediated by the ERK/MSK pathway and requires the coincident activation of dopamine D1 and glutamate NMDA receptors (Brami-Cherrier et al., 2005).

The effects of chronic cocaine are quite different for c*fos* and *fos*B. Thus, chronic doses also induce *fos*B but to a lesser extent (McClung et al., 2004) and via involvement of H3 acetylation. Malvaez et al. (2011) found that chronic cocaine led to reduced (but did not abolish) CBP occupancy and less acetylation at the c*fos* promoter compared with acute cocaine.

The switch from H4 acetylation with acute cocaine to H3 acetylation at the *fos*B promoter with chronic cocaine was a major finding and applies to many other genes (Kumar et al., 2005). The continued induction of *fos*B enables the spliced variant ΔFosB to accumulate as shown in Figure 8 in the preceding article.

The ability of cocaine to induce histone modifications at the c*fos* promoter, and several other genes, is greatly reduced after repeated cocaine exposure (Kumar et al., 2005; Malvaez et al., 2011), and often reported to be completely nullified (Kumar et al., 2005). Similar findings of reduced histone acetylation and histone methylation at the c*fos* promoter were reported for amphetamine (Renthal et al., 2008). c*fos* expression tends to be repressed partly because ΔFosB binds to its promoter and recruits an HDAC and an HMT (Renthal et al., 2008). Chronic cocaine may also augment histone acetylation through the phosphorylation and inhibition of HDACs which normally deacetylate and repress genes (Renthal et al., 2007). It is noteworthy that histone acetylation does not always lead to increased mRNA expression (Nestler, 2014).

3.1.2 G9a

The histone methyltransferase G9a catalyses the dimethylation of H3 at the lysine residue K9, (symbolically H3K9me2) which represses transcription. After acute cocaine, G9a is bound to H3 at the *fos*B promoter, thus inhibiting, but not blocking, cocaine induction of the *fos*B gene (Laplant & Nestler, 2011) which leads to less production of FosB and ΔFosB. Heller et al. (2014) showed directly that G9a induced H3K9me2 repressed *fos*B expression. G9a/GLP and PCR2 are two major epigenetic silencing machineries, which methylate histone H3 on lysines 9 and 27, respectively (Mozzetta et al., 2014).

There is an interesting feedback loop with ΔFosB and G9a. As stated above, acute cocaine G9a mediated methlyation occurs at the *fos*B promoter which reduces *fos*B expression. However, as ΔFosB accumulates, it represses the expression of G9a, which reduces the H3K9me2 levels of the *fos*B promoter. Thus, fosB expression tends to increase, resulting in more ΔFosB (Maze & Russo, 2010; Laplant & Nestler, 2011).

Repeated cocaine treatment reduces G9a and GLP expression and the enrichment of H3K9me2 in the NAc, resulting in derepression of the *fos*B gene (Maze & Russo, 2010; Heller et al., 2014). The suppression of G9a is mediated by ΔFosB (Nestler, 2013).

On the other hand, chronic drug treatment was found to recruit the histone deacetylase HDAC1 and G9a to

the c*fos* promoter, resulting in less acetylation and increased K9 methylation with the consequence of repression of the c*fos* gene (Laplant & Nestler, 2011). ΔFosB may also be involved in the repressive complex which leads to strong histone methylation at the c*fos* promoter (Robison & Nestler, 2011) as illustrated in **Figure 3**, adapted from figure 4(b**)** in the latter publication.

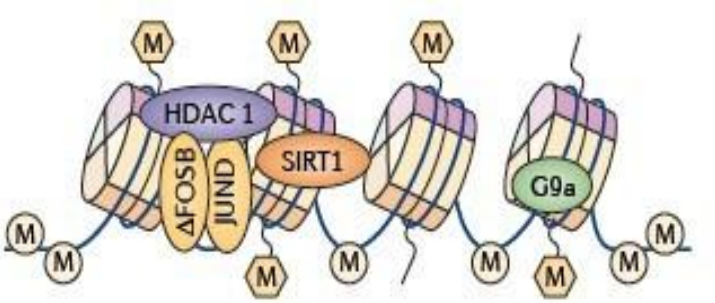

Figure 3 Some of the mechanisms involved involved in the repression at the c*fos* promoter after chronic drug application. Ms contained in hexagons indicate methylation. ΔFosB and JunD form a heterodimer which recruits the deacetylases HDAC1 and the sirtuin SIRT1. Histone methylation is also due to G9a, shown here at a nearby nucleosome. Adapted from Robison & Nestler, 2011.

Interestingly, chronic cocaine followed by 28 days of withdrawal, reduces H3K9me2 at the *fos*B gene, leading to increased induction of *fos*B in NAc (but not CPu). This was manifest as greater acute induction of ΔFosB mRNA and more rapid accumulation of ΔFosB protein after repeated cocaine reexposure (Damez-Werno et al., 2012).

Table 1: Histone modifications by residue type

Residue	Modifications
Arginine (R)	Methylation
Glutamate (E)	ADP-ribosylation
Lysine (K)	Acetylation
	Methylation
	SUMOylation
Serine (S)	Phosphorylation
Threonine (T)	Phosphorylation

Such modifications are reversible and can occur alone or in combination with others on the same or different histone tails. As there are some approximate rules for the effects of various modifications and combinations, the idea of a histone "code" emerged (Strahl & Allis, 2000). The same modification may have a different effect in different circumstances, such as mitosis or transcription. There may be interaction between modifications at different residues on the same or different histones, which could explain the variations in effect of a particular modification. For example, phosphorylation at serine 10 leads to chromatin condensation during mitosis and transcription in IEG induction (Strahl & Allis, 2000).

A well-documented case of synergism is the coupling of H3 phosphorylation of Ser10 (H3S10P) to acetylation at Lys14 (H3K14Ac) (Cheung et al., 2000). **Figure 4** illustrates this and further examples of synergistic and antagonistic crosstalk between modifications. There are several excellent accounts of epigenetic mechanisms including details of histone tail

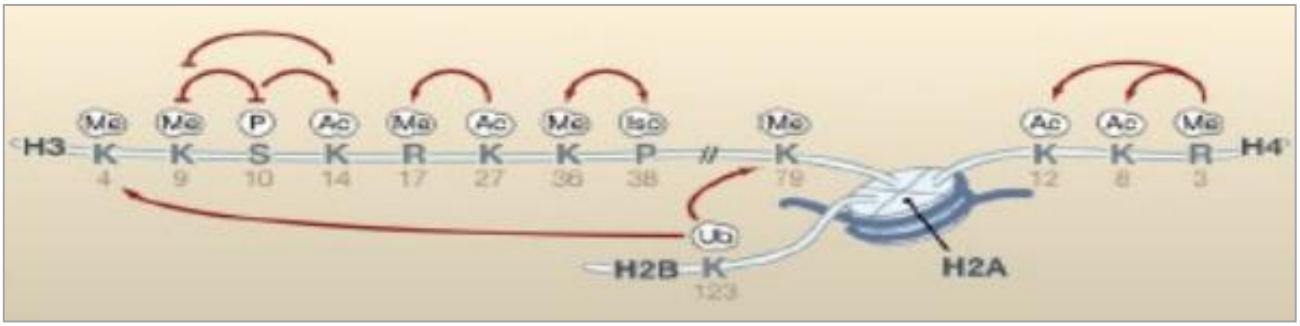

Figure 4: Example of Histone modifications and their interactions on tails of H3, H4 and H2B. The arrows represent cooperativity whereas curves ending with a bar indicate antagonism. Adapted from Kouzarides (2007).

3.2 Histone code

The Histone modifications mentioned above can be summarized (Berger, 2002; Nestler, 2014) as in **Table 1.**

amino acid structure and patterns of modification (Cheung et al., 2000; Strahl & Allis, 2000; Nowak & Corces, 2004; Li et al., 2007; Tsankova et al., 2007; Alberini, 2009; Maze & Nestler, 2011; Sawicka & Seiser, 2012; Nestler, 2016) including a study of the effects of

the antipsychotic medication on modifications at some sites (Li et al., 2004). The near arithmetic progression of the acetylatable lysine residues 9,14, 18 and 23 on H3 and 5, 8, 12 and 16 on H4 have been noted (Strahl & Allis, 2000).

3.2.1 DNA methylation

DNA methylation involves adding methyl groups to cytosine bases and is mediated by DNA methyltransferases (DNMTs). Its effect on gene transcription is generally repressive (Anier et al., 2010; Robison & Nestler, 2011; Nestler, 2014). DNMTs have been postulated to be involved in cognitive disorders such as schizophrenia.

Anier et al. (2010) conjectured that cocaine activation of the Ras signaling pathway could lead to both DNA methylation and histone modifications. They cite evidence that methylation of DNA brings about general deacetylation of histones H3 and H4 and prevents or induces methylation at other histones. With cocaine administration, in the NAc repressive complexes induced by administration of cocaine involving methyl CpG binding protein 2 (MECP2), for example, may be recruited at some methylated DNA sites such as the PP1c promoter which therefore downregulates PP1c.

However, in the NAc, at the *fos*B promoter, both acute and chronic cocaine lead to reduced methylation and decreased MECP2 binding which gives rise to increased transcription (Anier et al., 2010). Thus, MECP2 can function as a repressor or activator of transcription (Maze & Nestler, 2011). Within 20 minutes, acute administration of cocaine causes phosphorylation of MECP2 in the rat striatum and NAc, thus preventing it from functioning as a transcriptional repressor (Mao et al., 2011; Nielsen et al., 2012).

4. Pathways to Transcription

For transcription to occur, the promoter elements must be accessible to transcription factors and this requires that chromatin be in a decompacted state. Histone modifications, mainly phosphorylation and acetylation, have been strongly implicated as agents by means of which the chromatin is remodeled but there are many other proposed adjunct remodeling complexes. Key questions which have not yet been completely resolved concern what triggers the histone modifications and what is the temporal relationship between such changes and transcription. Many investigators have noted the close resemblance between the time courses of histone modifications, such as acetylation and phosphorylation, and transcription (for example, Cheung et al., 2000). The rather puzzling impression is given that these processes are almost simultaneous. In contrast, it seems that the order of steps should be histone modification followed by chromatin decompactification and then transcription, which begs the question of what initiates these processes. It was stated in Crosio et al. (2003), citing Cheung et al. (2000) and Berger & Felsenfeld (2001), that histone modifications can elicit chromatin remodeling, which is an *essential prerequisite* for the activation of IEG transcription. This was also the tenet of Brami-Cherrier et al. (2009): decompaction and change into (active) euchromatin is required for transcription to occur because it facilitates the operation of the core transcription machinery and the binding of transcription factors to promoter elements.

4.1 Overall Schemes

In this section, we first give examples of general schemes representing the main steps that lead from stimulus to transcription. We then give a more detailed description of some such schemes. Transitions in the state of chromatin-promoter systems that lead to transcription have been depicted by various authors, as exemplified by the following three reports.

4.1.1 Tsankova et al. (2007)

The general scheme described by Tsankova et al (2007) is depicted in **Figure 5**. The basic steps from an inactive promoter to an active one is according to Eqn (1):

$$\text{Inactive} \leftrightarrow \text{Repressed} \leftrightarrow \text{Permissive} \leftrightarrow \text{Active} \qquad (1)$$

where the steps are depicted as possibly proceeding in both directions.

In the inactive state, nucleosomes are tightly packed, and the associated histones are mainly methylated (M) or have associated repressor complexes (Rep). In the repressed state, there is some decompactification of the nucleosomes and a small elevation in the degree of histone acetylation (A). Further acetylation leads to the permissive state, and then with

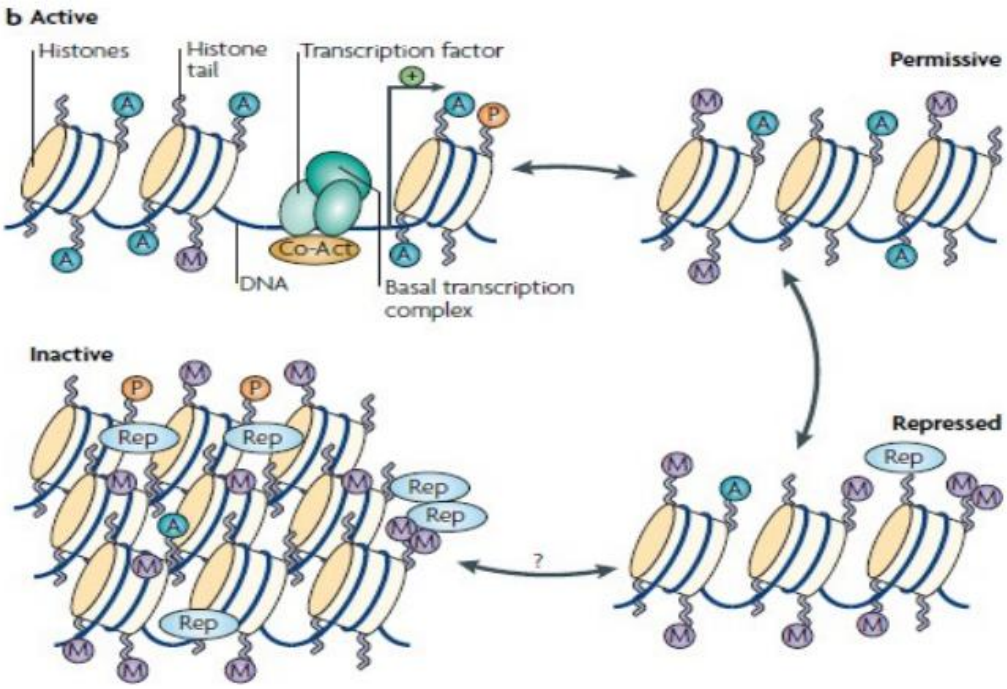

Figure 5 The scheme of Tsankova et al. (2007) showing the main states of nucleosomal structure from inactive to active.

more acetylation and phosphoacetylation, the transition to the active state of the gene occurs with the recruitment of transcription factor complexes (Co-Act).

4.1.2 Brami-Cherrier et al. (2009)

Here, the overall or core steps to transcription are given according to Eqn (2):

$$\text{Inactive} \rightarrow \text{Histones modified} \rightarrow \text{Active} \qquad (2)$$

In this scheme, which is also general but relates to specific histone modifications, the steps are unidirectional but the states are in a a cyclic loop as depicted in **Figure 6**. The inactive promoter again has compacted nucleosomes. After a stimulus that activates signaling pathways which will lead to transcription, H3 kinase phosphorylates H3 at Ser10 and a HAT acetylates H3 at K9/14 and H4 on K5. These events foster the decompactification of nucleosomes and hence enable the activation of transcription. The subsequent return of the inactive state is effected via dephosporylation of histones via phosphatases and deacetylation via HDACs.

4.1.3 O'Donnell et al. (2012)

In this example transcription was described as involving the distinct steps of (a) chromatin modification, and (b), the engagement of RNA polymerase and associated proteins involved in the transcription process. As shown in **Figure 7**, the basic steps to and from an active promoter are as in Eqn (3):

$$\text{Basal(repressed)} \rightarrow \text{Active} \rightarrow \text{Repressed} \qquad (3)$$

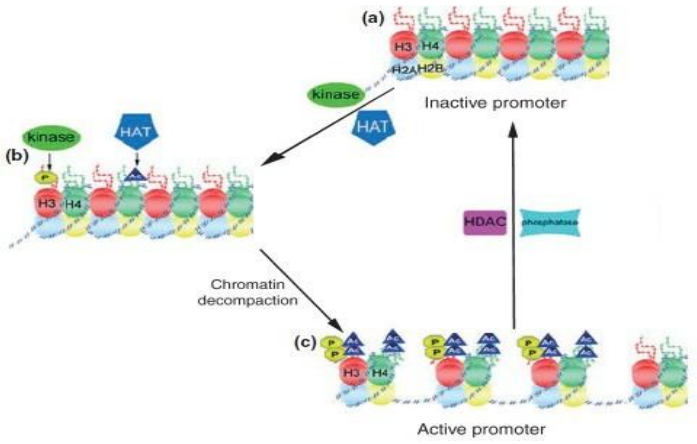

Figure 6 The scheme of Brami-Cherrier et al, (2009) showing the transition from inactive to active promoter via histone phosphorylation (P) and histone acetylation (Ac). The reverse process is shown accompanied by deacetylation and dephosphorylation. H3, H4, H2A, H2B, histones; HAT, histone acetylase; HDAC, histone deacetylase.

The focus is on unidirectional transitions when the c*fos* promoter is activated after the application of PMA (a potent activator of the SRE); CREB and the CRE are not involved in the scheme. In the figure ERK status is shown along with mRNA and chromatin status.

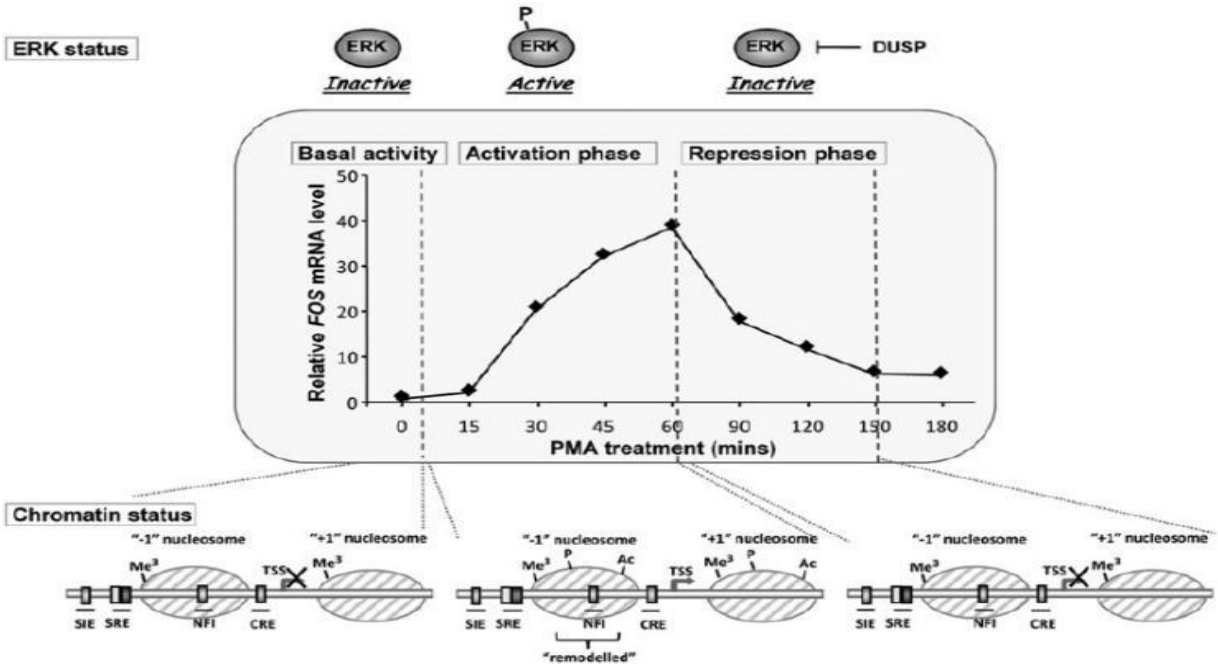

Figure 7: The scheme of O'Donnell et al., (2013). ERK status is indicated in the top part, showing phosphorylation (P) during the active phase and DUSP dephoshorylation at the inactive phase. The nucleosomes around the c*fos* promoter are designated -1 (upstream of the transcription start site, TSS) and +1, referred to as downstream. Prior to acti- vation, H3K4 is trimethylated (Me3). Activation is accompanied or triggered by H3S10 phosphorylation (P) and H3K14 acetylation (A) whereas deactivation (repression) results from dephosphorylation and deacetylation of these histone residues.

4.2 Details of steps to transcription

Outlines of histone modifications and transcription factor activation are given for the following three references.

4.2.1 Clayton et al. (2000)

These authors investigated the histone modifications at H3 in IE genes such as c*fos* and c*jun* in cultured mouse fibroblasts with techniques that recognized the simultaneous occurrence of phoshorylation and acetylation. They outlined the following steps in the transcription of such genes:

(1) A gene is primed by the association of some transcription factors such as TCF proteins (for example Elk1)

(2) MAPK (ERK) phosphorylates these transcription factors

(3) HATs such as CBP are recruited

(4) Nearby nucleosomes are thus acetylated

(5) H3 is phosphorylated by MSK1 which is activated by ERK

and acetylated upon gene activation. The phosphorylation occurs on Ser10 and the acetylation on Lys9 but other modifications including methylation probably also occur. These authors noted that MAP kinases, which are involved in the phosphorylation of transcription factors also mediate the phosphorylation of H3. Thus, the same signaling cascades are instrumental in both activating transcription factors and mediating the nucleosomal response so that these two responses are linked. Furthermore, ChIP assays demonstrated that H3 is also phosphoacetylated at c*jun* promoters upon MAP kinase pathway activation (Cheung et al., 2000; Clayton et al., 2000).

4.2.2 Crosio et al. (2003)

Experiments were performed by Crosio et al. (2003) in which transcriptional activity and H3 phosphorylation were analyzed in mouse hippocampal neurons on the intraperitoneal administration of agonists of dopamine receptors, muscarinic acetylcholine receptors and kainate glutamate receptors. c*fos* expression was detectable at 15 minutes with a maximum of about 1 hour. Induction of H3 phosphorylation seemed to always *precede* c*fos* transcription. The interval between these events is probably a few seconds (*E.J. Nestler, personal*

communication) so that they may appear practically simultaneous. It was also established that the time course of histone H3 phosphorylation paralleled the *concomitant* phosphorylation of the ERKs and that these events occurred in the same neurons. In addition, the time course of phosphorylation of histone H3 "correlates" with the induction of the IEGs such as c*fos*. Phosphoacetylation of H3 occurred in response to the application of the neurotransmitter receptor agonists, and it was deemed likely that phosphorylation occurs preferentially on unmodified H3 and that a fraction of the phosphorylated H3 is subsequently also acetylated. Rather confusing is that high levels of acetylation were found on both Lys9 and Lys14 in saline-treated animals and "bulk levels" of H3 acetylation did not significantly change after treatment. It was reasoned that this occurred because acetylated H3 is generally associated with active chromatin.

4.2.3 Brami-Cherrier et al. (2009)

However, Brami-Cherrier et al. (2009) distinguished two schemes concerning the relationship between phosphorylation and acetylation of H3. The first is the "synergistic" model whereby phosphorylation primes H3 for acetylation before transcription occurs. In the "parallel" model, phosphorylation occurs on pre-acetylated histone H3 to lead to IEG transcription, the phosphorylation and acetylation being independent. In neurons of the striatum and hippocampus there was evidence in support of the parallel model.

In the context of IEG induction, exemplified by c*fos* and c*jun*, these authors, citing Mahadevan et al. (1991), Chadee et al. (1999) and Thomson et al. (1999), pointed out that H3 phosphorylation at Ser10 and acetylation at Lys14 at the promoters of these genes were *necessary and sufficient* for the nucleosomal response (rapid phosphorylation of H3) and that these modifications occurred on the same histone tail. The phosphorylation of H3 on Ser10 involves the MAPK/ERK signaling pathway which leads to the activation of RSKs and MSKs by means of complicated sequences of phosphorylation. MSK1 is of key importance in H3 phosphorylation and c*fos* and c*jun* induction by glutamate in striatal neurons.

Of interest, is that both RSKs and MSKs *target not only H3 but also the transcription factor* CREB, as shown in Figures 3C and 3D of our introductory article (Tuckwell, 2019), which could partly underly the similar time courses of H3 modification and IEG induction. Noteworthy also is that evidence points to infrequent PKA involvement in H3 phosphorylation, although Li et al. (2004) found that D2 receptor antagonists such as certain antipsychotics induced H3 phosphoacetylation by cAMP-dependent PKA and postsynaptic NMDA receptor signaling. Further, MSKs are more effective as H3 kinases than RSKs and their distribution is intense in neurons, especially of the striatum and the amygdala. The phospatases PP1 and PP2A have both been implicated in the direct dephosphorylation of H3. PP2A may also participate in H3 dephosphorylation by inactivating the H3 kinases.

5. Transcription in single cells: stochastic bursting

The experimental data on mRNA and protein levels described above appear smooth as depicted in Figures 5, 6 & 9 in Tuckwell (2023). However, such results represent contributions from populations of large numbers, possibly millions, of cells which masks the stochastic nature of the transcription events occurring in single cells (Cesbron et al., 2015; Wang et al., 2019).

5.1 mRNA recordings in single cells

An early study of c*fos* mRNA in single cells was that of Huang & Spector (1991) who tracked the production and subsequent movement of mRNA molecules through nuclear pores to the cytoplasm. There was a transient rise of c*fos* mRNA in response to serum and after 15 minutes mRNAs appeared in the cytoplasm.

Given that the underlying chemical reactions via signaling pathways leading to activation and possible translocation of transcription factors together with the decompactification of DNA as well as the setting up of the transcription machinery around RNA polymerase are complicated (Suter et al., 2011; Tantale et al., 2016; Chen et al., 2019) and with components that have uncertain concentrations, stochasticity is expected to be prevalent in gene transcription in single cells. A very good summary of the complicated molecular reactions involved in transcriptional bursting is contained in Wang et al. (2019). In the simplest approximation, if the underlying mechanisms are construed to be constitutive, resulting in a steady process with small variance, in which single mRNA molecules are produced randomly with a constant mean rate, then a

temporally homogeneous Poisson process (Tuckwell, 2018) may be used as a model. Mathematically this description has been called a one-state model (Senecal et al., 2014; Corrigan et al., 2016; Wang et al., 2019), the time interval between events having an exponential distribution. An interesting review of the earlier historical stochastic aspects of gene expression was given by Paulsson (2005). Tutucci et al. (2018) contains an excellent review of more recent theories.

Jaenisch & Bird (2003) describe the combined effects of DNA methylation, histone modification and chromatin remodeling as a complex process with many epigenetic components. Dynamic transitions between different chromatin states are determined by the balance between factors that sustain a silent state (including HDACs) and those that promote a transcriptionally active state (including HATs). Perturbations of any of these components may shift the balance between active and silent chromatin states with resulting in increases or decreases in the chance of transcription. Another relevant chromatin modification consists of nucleosome sliding, increasing access to DNA binding sites through protein complexes such as SWI/SNF (Sng et al., 2004; Tsankova et al., 2007).

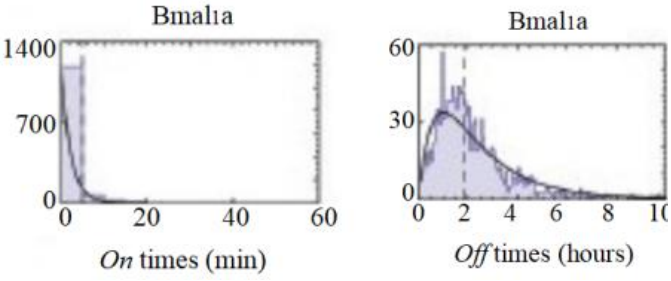

Figure 8 Histograms of on and off periods of mRNA production in a mammalian gene Bmal1 with fitted curves. Adapted from Suter et al. (2011a).

It has been found that transcription in individual eukaryotic cells and bacteria proceeds in bursts, which may produce 1 to 50 transcripts (Vera et al., 2016) per burst. Thus, for many genes, mRNA molecules are formed in short bursts during an ON or active period, whereas between ON periods are OFF or inactive periods in which there is no or perhaps very little mRNA production. Both ON and OFF periods appear to start and end randomly. ON periods usually have durations of order minutes, whereas the duration of OFF periods is of order a few hours (Cesbron et al., 2015; Suter et al., 2011b; Wang et al., 2019). An example of distributions of on and off times is shown in **Figure 8** where on periods are of much less duration than off periods. However, this is not always the case as seen in data of Larson et al. (2013), whereby increasing stimulus strength made the duration of off times decrease until it became similar in magnitude to that of the on times, although the latter remained fairly constant. The decrease in the length of off periods occurred because the stimulus was effective in promoting acetylation of histones, which, along with other chromatin modifications promotes transcription (Raj et al., 2006; Cesbron et al., 2015; Nicolas et al., 2018; Tutucci et al., 2018; Chen et al., 2019).

Transcription events can thus be construed as a continuous time stochastic process which in a popular form has often been described as a two-state model or random telegraph signal (Peccoud & Ycart, 1995; Raj et al., 2006; Larson et al., 2009; Suter et al., 2011a; Dar et al., 2012; Larson et al., 2013; Senecal et al., 2014; Tantale et al., 2016). The scheme of the model is shown in **Figure 9**. There are three parameters which describe three point processes in the two state model, being the rate λ_{on} of switching to the ON state, the rate λ_{off} of switching back to the OFF state and the rate of production λ_M of nascent mRNA during the ON periods. The parameter d_M is the rate of degradation of mRNA molecules, usually considered to be cytoplasmic though nuclear retention can sometimes be a factor (Bahar-Halpern et al., 2015). In relation to bursts the following random quantities are of interest and the factors which modulate or control them. The burst frequency, which is the number of ON periods per unit time, the burst size, which is the number of mRNA molecules released per burst and the burst duration which is the time between OFF periods.

Analytical expressions have been obtained for the statistical properties of time dependent and steady state mRNA numbers by several authors including Peccoud & Ycart (1995), Raj et al. (2006), Iyer-Biswas et al. (2009), Larson et al. (2009), Shahrezaei & Swain (2009) and Dattani et al. (2017).

The above two-state model has been extended to include the random production of protein (translation) with rate parameter λ_P and its subsequent degradation at rate d_P; see for example Peccoud & Ycart (1995),

Raj et al. (2006) and Larson et al. (2009). Thus one distinguishes between transcription noise and translation noise (Kœrn et al., 2005; Tutucci et al., 2018). Further extensions concern the inclusion of transitions between inactive and active promoter states (Shahrezaei & Swain, 2008; Chen et al., 2019), the adding of additional OFF states (Suter et al., 2011a; Wang et al., 2019) and the inclusion of a continuum of transcriptional states (Corrigan et al., 2016). Experimental findings, including RNA statistics for c*fos* and a computational model called the finite state projection approach are contained in Munsky et al. (2015).

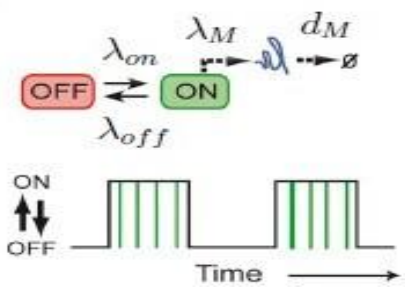

Figure 9 Schematic for the two-state telegraph model for mRNA bursts. During OFF periods no mRNA is produced whereas in the ON periods mRNA molecules are produced in batches at a Poisson rate of λ_M. Such molecules eventually decay to the nondescript state ϕ. Adapted from Senecal et al. (2014).

5.2 Results for c*fos*

Of special relevance to understanding the dynamics of c*fos* expression are results such as those of Senecal et al. (2014) for measurements of c*fos* mRNA numbers in single cells. Stimuli employed were serum and zinc which both activate the MAPK pathway and activate the promoter at the SRE element. **Figure 10** shows results for both mRNA numbers per cell and levels of the transcription factor phosphorylated ERK as functions of time with serum stimulation. A fluorescence procedure called smFISH was used to obtain mRNA numbers and quantitative reverse transcription polymerase chain reaction (qRT-PCR) provided a verification. The mRNA level rose to a maximum at 30 minutes and declined to basal values at two hours. According to Senecal et al. (2014), p-ERK quickly accumulated in the nucleus, reaching a maximum at 10 minutes and declining to near-basal values after 1 hour. The rise of p-ERK to its peak (in 10 minutes) and its decline to 50% of peak value (in 33 minutes) seem about twice as rapid as the rise and decline of mRNA.

Some other very interesting results in Senecal et al. (2014) are as follows:

(i) Concentration of transcription factors determines the burst frequency. In the case of c*fos*, p-ERK level correlated with the burst frequency.

(ii) Bursts last three to four minutes.

(iii) During a burst, several mRNAs are produced per minute with total numbers per burst of order several tens.

(iv) The time taken to construct a single mRNA is about 1 minute.

(v) Burst sizes are fairly constant regardless of their frequency and are independent of transcription factor concentration (assuming this is suprathreshold).

(vi) At maximal mRNA production (20 minutes), a second ON state would explain the data better than the simple two-state model.

(vii) Burst duration depends on the duration of transcription factor (complex) binding to promoter elements.

Chen et al. (2019) also examined the dynamics of c*fos* transcription in cultured embryonic mouse cortical neurons at the single cell level using depolarization with externally applied KCl, mimicking the effects of Ca^{2+} influx from synaptic input. A five minute depolarization was sufficient to cause increases in c*fos* mRNA and acetylation at H3K27. Such increases were transient and had time courses similar to those in the study of Senecal et al. (2014). Using previously developed mathematical techniques, five parameters related to c*fos* transcriptional bursting were estimated, including λ_{on}, λ_{off}, λ_M, the rate of mRNA production in the OFF state and the time interval between initiation of transcription and the formation of nascent cytoplasmic mRNAs. The value of the parameter d_M was obtained from Shyu et al. (1989). Promoters were in the ON state for an average of 11 minutes. The duration of OFF states was 100 minutes before the depolarizing stimulus, being reduced to just 4 minutes after depolarization whereas the ON state duration was unchanged. Chen et al. (2019) also studied the positive role of acetylation of enhancers located on loops that come into close contact with promoters.

Munsky et al. (2015) obtained a very interesting set of histograms for the numbers of nascent c*fos* mRNA induced by serum in human bone cancer cells (U20S) at various times. At 10, 20, 30 and 45 minutes the ranges of mRNA counts were [2,6], [2,17], [2, 13] and [2, 7] respectively, having qualitatively similar time courses to many macroscopic results. The distribution at 10 minutes was roughly Gaussian, with roughly low order gamma-like distributions at the subsequent time points.

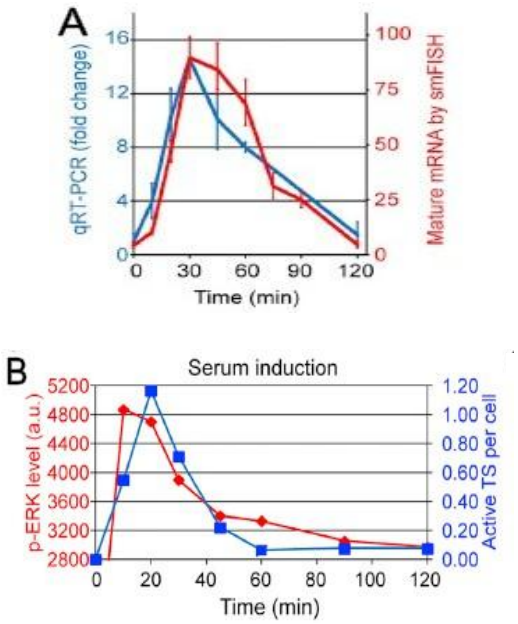

Figure 10 Results for serum stimulation. A. Mean c*fos* mRNA levels from single cell smFISH analysis (red) and qRT-PCR measurements (blue). B. Corresponding results for phosphorylated ERK (red) and active transcription sites per cell (blue). Adapted from Senecal et al. (2014).

6. Discussion

The sets of biochemical reactions called signaling pathways, which are set in motion by activation of receptors such as NMDA and D1 in medium spiny neurons of the striatum, are numerous and complex. Most of these pathways involve sequences of phosphorylation reactions, which usually enable, whereas dephosphorylations are usually disabling. The result of the activation of the relevant pathways is the activation of transcription factors, which may bind (or already be bound but inactive) to promoter elements at DNA segments to instigate the production of mRNA. In addition, the transcription machinery must be assembled, which usually involves the recruitment of many molecules. An important step in transcription is unraveling nucleosomes to make the promoters accessible to the transcription machinery and the transcription factors. The complexity of the biochemical reaction schemes, which involve cytoplasmic and nuclear components, implies that transcription is a multidimensional stochastic process, as evidenced by results for single cells. The experimental foundations of such processes are being unfolded in many laboratories. Several relevant results are contained in the present sequence of papers. Many excellent deterministic and stochastic models have been advanced to describe the components of signaling pathways in striatal and hippocampal neurons. These have been or will be mentioned at various places in the texts.

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