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Nucleocytoplasmic shuttling of transcription factors

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Abstract. To elicit the transcriptional response following intra- or extracellular stimuli, the signals need to be transmitted to their site of action within the nucleus. The nucleocytoplasmic shuttling of transcription factors is a mechanism mediating this process. The activation and inactivation of the transcriptional response is essential for cells to progress through the cell cycle in a normal manner. The involvement of cytoplasmic and nuclear accessory molecules, and the general nuclear membrane transport components, are essential for this process. Although nuclear import and export for different transcription factor families are regulated by similar mechanisms, there are several differences that allow for the specific activation of each transcription factor. This review discusses the general import and export pathways found to be common amongst many different transcription factors, and highlights a select group of transcription factors that demonstrate the diversity displayed in their mode of activation and inactivation.

Key words. Transcription factors; nuclear shuttling; export; import; cell cycle.

Introduction

A major barrier for proteins to overcome as they are newly synthesized in the cellular cytoplasm is that of finding their correct final destination within the cell. The complex systems of membrane transport have been widely studied, and those of the nuclear membrane are no exception. For all transcription factors, crossing this nuclear/cytoplasmic barrier is an essential process that must be carried out efficiently as the cell responds to various intra- and extracellular stimuli. For proteins destined for the nucleus, the transport takes place through structures within the nuclear membrane known as nuclear pores. These pores are composed of large multiprotein complexes of the order ~125 MDa termed nuclear pore complexes (NPCs), which have been relatively well conserved throughout evolution. A detailed analysis of this structure is beyond the scope of this review and has been recently well described [1]; however, a brief outline of the soluble components required for transport across the NPC is required since it is at this level that specificity is controlled.

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It is now accepted that both soluble cytoplasmic and nuclear components are required together with the NPC to facilitate active protein transport into and out of the nucleus. This active nucleocytoplasmic transport process is mediated by the small GTPase (GTP hydrolysing enzyme) Ran [2–4]. Ran is a protein which is associated with GTP in the nucleus and GDP in the cytoplasm [3]. The Ran cycle of GTP hydrolysis requires the involvement of cytoplasmic accessory molecules such as a GTPase-activating protein (Ran-GAP1) [3] and a Ran-binding protein (RanBP1) [5], which generate inactive GDP-bound Ran. Ran-GDP is required to form the nuclear import complex with the cargo molecule and following translocation requires nuclear accessory molecules such as RCC1, which mediates the nucleotide exchange of GDP for GTP (fig. 1). The transport of Ran through the NPC into the nucleus is dependent on another molecule termed NTF2. NTF2 (nuclear transport factor 2) was identified as a factor which stimulates the import of proteins containing a nuclear localization signal (NLS) [6]. NTF2 only associates with the GDP-bound form of Ran and not the GTP-bound form. This lack of affinity of NTF2 for Ran-GTP allows for their complex disruption in the nucleus following nucleotide exchange to generate the Ran-GTP state [7]. This GDP/

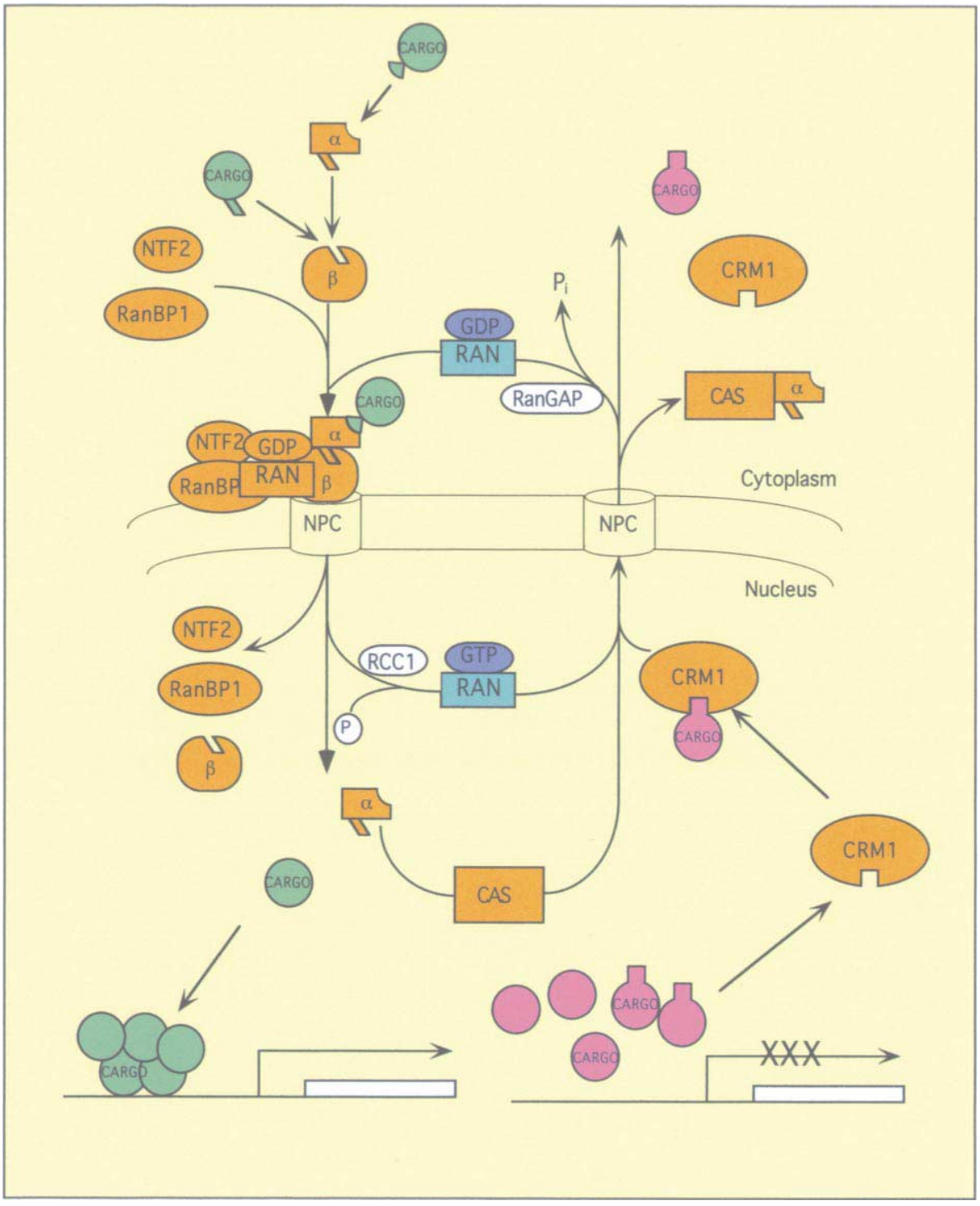

Figure 1. Overview of the general nuclear/cytoplasmic import/export pathway. α, importin-α; β, importin-β. See text for further details.

GTP cycle is a key feature for all current models for active nucleocytoplasmic shuttling, and disruption of this cycle generates inhibition in nuclear transport; however, it is becoming clear that the energy resulting from the hydrolysis is not the regulating mechanism behind this shuttling control [8, 9]. The control is most likely generated by the specificity of Ran-GDP for importing cargo, Ran-GTP for exporting cargo, and the NPC-importin and NPC-exportin interactions.

Further components facilitating nuclear transport include a group of carrier proteins that feature a family of proteins termed importins/transportins and exportins. One of the main features of this family of proteins is that they interact with Ran through an amino-terminal binding domain. These proteins are responsible for interaction with the cargo molecule and docking of the complex to the NPC; however, they are not essential for all transported molecules. The importins are known as importin-α and -β. Importin-α recognizes and binds to specific signals on the cargo molecule termed the nuclear localization sequence (NLS) through its specific NLS binding region. This complex is enhanced by the binding of importin-β through the importin-β binding domain (IBB domain) of importin-α [10], which then mediates the docking of the import complex to the NPC. Nuclear export of importin-α is facilitated by CAS (cellular apoptosis susceptibility gene), which preferentially interacts with importin-α in the presence of Ran-GTP [11] and hence allows recycling of the import carrier. Alternatively, proteins possessing IBB-like NLS sequences can bypass the requirement for importin-α and interact directly with importin-β [12, 13] (fig. 1). The molecular mechanisms involved in active nuclear transport are becoming clearer following the recent publications of the structures of importin-β bound to the IBB domain of importin-α [14] and importin-β bound to Ran [15, 16].

Because most proteins targeted for the nucleus via the importin pathway possess an NLS corresponding to either the single-site motif, such as in the SV40 large T antigen [17], or the bipartite motif found in nucleoplasmin [18] (table 1), it is also becoming clear that proteins requiring active removal from the nucleus require the presence of a nuclear export signal (NES). The NES consensus sequence was first identified for the cyclic AMP (cAMP)-dependent protein kinase inhibitor, PKI, [19] and for the RNA-binding protein of the human immunodeficiency (HIV) virus, Rev [19, 20] (table 1). It was found to comprise a small leucine-rich region which was sufficient to target a fused protein efficiently out of the nucleus [19]. Following this identification of an NES motif, many proteins have been described as possessing potential NES sequences (table 1).

The major controlling element of the nuclear export process in addition to the RanGTP/GDP cycle is the protein Crm1/exportin1/Xpo1. Crm1 was originally identified as an essential nuclear protein in fission yeast [21] and was later shown to be essential for export of proteins from the nucleus in yeast [22]. In parallel, Crm1 was identified as a 110-kDa protein in *Xenopus* extracts, which bound to an intact leucine-rich nuclear export signal, and this interaction could be inhibited by the cytotoxic drug leptomycin B [23]. Leptomycin B has been described as being capable of direct interaction with Crm1, which then prevents NES-dependent nuclear export [24, 25]. Interestingly, leptomycin B is unable to block nuclear export in the yeast *Saccharomyces cerevisiae* [26], and it was demonstrated that this is most probably due to the fact that *S. cerevisiae* Crm1p fails to directly interact with leptomycin B [24].

In this review we have concentrated principally on the aspects of nuclear translocation (both import and export where possible) of some selected families of transcription factors. Each family has been extensively reviewed with regard to biological function. It is not our aim to cover the same ground, and the reader is referred to recent reviews for further details where appropriate. Here we have attempted to describe the regulatory mechanisms involved in nucleocytoplasmic shuttling for each family and present simplified models for each in order to highlight the diversity displayed by each family.

Table 1. Proteins contain either an NLS or NES, which targets them for nuclear import or export, respectively. Examples of some protein sequence motifs recognized as acting as NLSs or NESs are indicated. References are shown in square brackets

Proteins and their NLSs		
SV40 large T	PKKKRKV	[17]
Nucleoplasmin	KRPAAIKKAGQAKKKK	[18]
p14ARF	QLRRPRHSHPTRARRCP	[72]
E2F-1	PVKRRLDL	[33]
E2F-2	PAKRKLDL	[33]
E2F-3	PAKRRLEL	[33]
NF-AT (1)	CNKRKYSLN	[83]
NF-AT (2)	GKRKK	[83]
Proteins and their NESs		
hdm2	LSFDESLALCVI	[67]
mdm2	LSFDPSLGLCEL	[67]
p53	MFRELNEALELK	[64]
p73	ILMKLKESLELM	[64]
PKI	LALKLAGLDIN	[19]
HIV rev	LPPLERLTLD	[19, 20]
HTLV rex	LSAQLYSSLTL	[67]
Dsk-1	SLEGAVSEISLRD	[23]
MAPKK	ALQKKLEELELDE	[23]
NF-ATc	SAIVAAINALTT	[74]

The E2F family

The E2F family of transcription factors was originally identified as the proteins responsible for transactivation

of the adenovirus E2 promoter [27]. They were also found to be associated with members of retinoblastoma tumor suppressor protein family pRB, p107 and p130, collectively known as the pocket proteins (PPs) [28]. The E2F transcription factors are comprised of two major classes termed the E2Fs and the DPs. Within the E2F class there are six members, namely E2F-1 through -6, whereas only two members have been identified belonging to the DP class in humans. E2Fs 1–5 have been shown to possess transcriptional activation capabilities [29], whereas E2F-6/EMA (E2F-modulating activity) has been proposed as an inhibitor or repressor of transcription [30, 31]. In all cases of E2F-dependent transcription, each E2F component functions as a heterodimer with one member of the DP class. This heterodimer is termed the active E2F transcription complex. E2F has been described as being involved in the transcriptional regulation of many cell-cycle-regulated genes [29]. E2F targets are required for correct progression from the G1 phase to the S phase of the cell cycle, highlighting the importance of this complex for normal cell proliferation [29]. Accordingly, the deregulation of E2F activity leads to incorrect passage from early G1 to S, and components in this pathway are found mutated in many human cancers [29, 32]. The activity of E2F is regulated by various mechanisms [29], and this review focuses on intracellular localization as one such mechanism.

E2F-1, -2 and -3 are nuclear proteins due to the presence of a conserved amino terminal NLS, which is absent from the other members of the E2F class [33–35]. These three proteins are induced and accumulate in the nucleus in G1 and downregulated as cells progress through the S phase. E2F-6 is also a nuclear protein, although an NLS has not been directly demonstrated for this protein [31]. E2F-4 and -5, however, are found at relatively constant levels throughout the cell cycle and are regulated by their translocation to the nucleus, but they lack an obvious NLS. E2F-4 and -5 are predominantly cytoplasmic proteins that are localized to the nucleus in the G0 and early G1 phases of the cell cycle and relocalized to the cytoplasm in mid-G1, when E2Fs 1–3 become activated. The proposed mechanism for the translocation of E2F-4 and -5 to the nucleus involves the binding of a partner protein containing an NLS to mediate the transport. It was demonstrated that transfected wild-type E2F-4 is predominantly cytoplasmic, but when attached to an NLS from either E2F-1, E2F-2 or the SV40 large T antigen, it was predominantly nuclear, indicating that the lack of an accessible NLS in E2F-4 was preventing its nuclear translocation [33, 34, 36].

The members of the DP class have also been proposed to be responsible for translocating E2F members to the nucleus [35, 37–39]. Alone, ectopically expressed DP-1 is predominantly cytosolic; however, when expressed with E2Fs 1, 2, 3 or 6, it is translocated to the nucleus, suggesting that these members of the E2F family mediate its nuclear translocation [31, 37, 38]. Following disruption of the E2F/DP-1 complex, DP-1 is directed back to the cytoplasm, where it undergoes ubiquitination and proteasome-dependent degradation [40]. DP-2 on the other hand, possesses an NLS and is a nuclear protein which when coexpressed with E2F-4 or -5 mediates their nuclear localization [35, 38]. For E2F-4, results have demonstrated that the mere sequestering of the protein out of the nucleus is enough to render it inactive. When tethered to an NLS, not only does it translocate to the nucleus, but it has full transcriptional activity as determined using E2F-site dependent luciferase reporter constructs and is capable of driving cells from quiescence into S phase [34], as has been demonstrated for E2Fs 1–3. An alternative mechanism for nuclear translocation of E2Fs 4 and 5 has been ascribed to the pocket proteins p107 and p130 [35, 38]. This allows for an extra level of control in the activity of the E2F family, since nuclear translocation due to pocket protein binding will generate a negative effect on cell cycle progression, whereas DP-mediated nuclear accumulation will induce cell growth (fig. 2).

In summary, several levels of nucleocytoplasmic shuttling of members of the E2F transcription factor family have been described; however, the underlying mechanisms involved in shuttling have not been well resolved. An involvement of importins, exportins or the RanGTP cycle has not been demonstrated for any E2F protein. It is yet to be determined whether nuclear export of E2F proteins is an energy-dependent process or whether it is driven by the unmasking of an intrinsic NES in some members. The targeting of E2Fs for degradation may also be a driving force for nuclear export. The importance of the E2Fs in maintaining accurate progression through the cell cycle make these unresolved issues a focus of future research to provide a better understanding of E2F function.

The REL/NFκB/IκB superfamily

The nuclear factor κB (NFκB) family members have been reported as playing a major role in the transcriptional control of many pro- and antiapoptotic genes such as tumor necrosis factor α (TNF-α), interleukin (IL)-2, granulocyte-macrophage colony-stimulating factor (GM-CSF), C-MYC and p53 [41]. They have also been identified associated with various other transcription factors such as AP-1 and Sp1, and the transcriptional coactivator p300/CBP [42]. NFκB members are involved in eliciting the transcriptional response following extracellular signaling through receptors such as IL-1R and TNF receptor (TNFR) and many target

genes are central to promoting an immune response. Their function in providing an antiapoptotic response following stimuli is counteracting the proapoptotic effects of p53 by competing for a limiting pool of p300/CBP, indicating there may be a possible equilibrium between these two molecules to provide positive and negative regulation for the cell [43].

The members of the NFκB group of transcriptional regulators are localized in the cytoplasm in their inactive states. There are five components of the NFκB

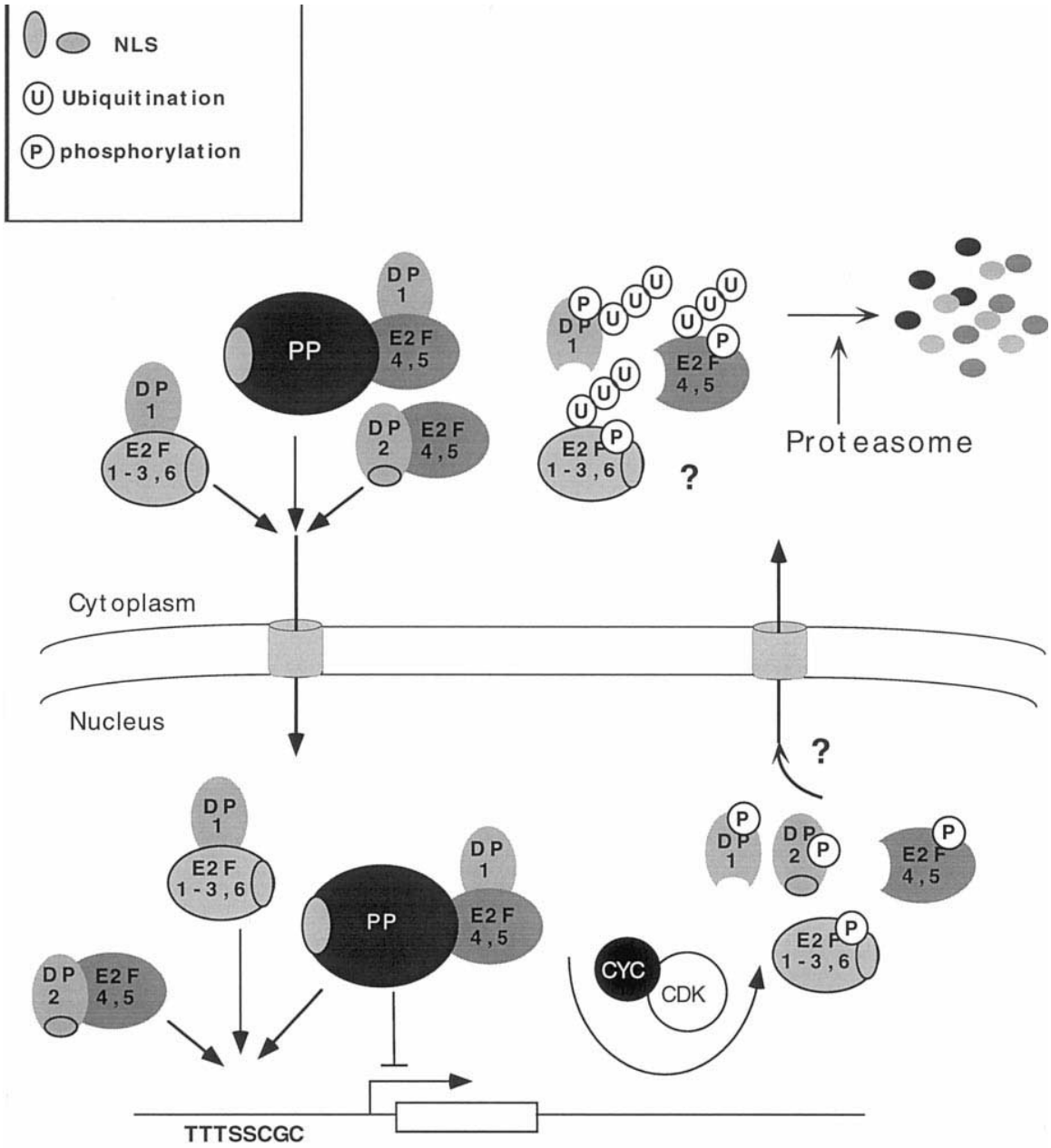

Figure 2. Nuclear shuttling of the E2F transcription factors. E2F members are imported into the nucleus via NLS sequences, presumably via importins. The ? indicates the mechanism of export is unresolved. Following phosphorylation by the cyclin/cdk complexes (CYC/CDK), the E2Fs are released from DNA, at which point the export/degradation takes place. The E2F-specific DNA binding sequence is indicated where SS is either a C or G. PP indicates members of the pocket protein family, namely pRB, p130 or p107. See text for further details.

subfamily, namely RelA (p65), c-Rel, RelB, p105/p50 (NFκB1) and p100/p52 (NFκB2), and they all possess a conserved 300-amino-acid motif termed the REL homology domain (RHD) [41]. It is through this conserved domain that dimerization, DNA binding, nuclear localization and inhibitor κB (IκB) binding take place. Both p105 and p100 are synthesized as precursor proteins, which are proteolytically processed by the proteasome to remove the carboxy-terminal domain to generate the active p50 and p52 subunits, respectively [44–46]. RelA, RelB and c-Rel all possess transactivation domains located within their carboxy termini, whereas p50 and p52 do not.

When cells are unstimulated, the NFκB dimers are maintained in a cytoplasmically localized inactive state sequestered by the IκB family members due to masking of the NFκB NLS. The IκB protein family is composed of five members, of which four are localized to the cytoplasm. These proteins (termed IκB-α, IκB-β, IκB-ε, IκB-γ and BCL3) contain a large domain comprising several ankyrin repeats [41]. BCL3 is a nuclear protein and serves to enhance the activation of NFκB, whereas the other four members are cytoplasmic and function as inhibitors. Sequestration in the cytoplasm is the major regulatory mechanism for the inactivation of NFκB. A complex of c-Rel and IκB-α is readily maintained in the cytoplasm, but loss of control by IκB-α results in oncogenic activation of c-Rel [47]. This cytoplasmic localization is ensured by the binding of the IκB-α subunit. The v-Rel oncoprotein is an activated version of c-Rel. v-Rel is activated by two point mutations in a region close to its NLS. These point mutants alter the affinity of v-Rel for IκB-α and expose the NLS in v-Rel. Thus, v-Rel is constitutively nuclear and active as a transcription factor [47]. Therefore, the requirement for cytoplasmic retention of the active components of the NFκB family is essential for normal cellular growth control.

Following TNF-α/FAS stimulation, IκB-α is phosphorylated on two amino terminally situated serine residues (ser32 and ser36) [48, 49]. The phosphorylation of IκB is mediated by a kinase cascade culminating in the release of IκB from the NFκB dimers followed by its degradation by the proteasome [50]. IκB-α can be phosphorylated directly and specifically on serines 32 and 36 by protein kinase CKII in vitro, suggesting a possible kinase responsible for IκB-α release [49]. However, the major kinase responsible for phosphorylating IκB is IKK (IκB kinase) [51–53] (fig. 3). IKK phosphorylates both IκB-α on serines 32 and 36 and IκB-β on serines 19 and 23. This kinase complex comprises IKK-α (IKK1) and IKK-β (IKK2) subunits, both of which possess amino-terminal kinase domains. Other components of the IKK kinase include the IKKγ/NEMO [54, 55] subunit, which binds the IKK complex as a dimer, the $\sim$50-kDa IKKAP1 (IKK-associated protein 1) subunit, which displays a specific interaction with the IKK2 component through a defined amino-terminal region of IKKAP1 [56], and the 150-kDa IKAP protein (IKK-complex-associated protein), a part of the IKK complex functioning in the assembly of the active IKK complex [57].

The pathway leading to degradation of IκB-α has been under intense investigation. Recent data suggest that the F-box protein β-TrCP is responsible for binding the phosphorylated form of IκB-α [58]. β-TrCP is part of an E3 ligase complex (Skp1/Cul1/β-TrCP), and the binding of β-TrCP to IκB-α thus targets the protein for ubiquitination and subsequent degradation. An alternative but parallel model for IκB-α removal has also been postulated, whereby IκB-α undergoes proteasome-independent degradation [59]. This was postulated due to the incomplete block in the degradation of IκB-α following the addition of proteasome-specific inhibitors. Following TNF-α stimulation, cytoplasmic calpains are activated which can function to degrade IκB-α, and this activation could be inhibited specifically by calpain inhibitors [59] (fig. 3).

All mechanisms leading to the degradation of the IκB family members generate the same downstream effect, that of activation of the NFκB dimers. Other pathways have been reported in which NFκB is activated without the degradation of IκB. This occurs in the case of IL-1 stimulation, which induces the phosphatidylinositol 3 kinase (PI3K). An increase in phosphorylation of the RelA subunit in particular is observed after IL-1 stimulation, and this phosphorylation can be blocked by specific PI3K inhibitors. Furthermore, expression of the active p110 subunit of the PI3K complex induces p65 phosphorylation and subsequent transactivation of NFκB [60]. The timing of p65 phosphorylation by PI3K is unknown, and whether it occurs in the presence or absence of IκB has not been determined. This pathway of NFκB activation appears to be a cytoplasmic event, after which nuclear translocation and DNA binding take place.

NFκB, once activated by IκB release, is targeted for the NPC. A complex with importins α and β is formed, and Ran-GTP/GDP-dependent nuclear translocation is initiated. A feedback loop exists for the NFκB activation/inactivation cycle. One of the gene targets of an active nuclear NFκB complex is that of IκB. As newly synthesized IκB accumulates in the nucleus, where it is inaccessible to phosphorylation [61], it sequesters NFκB and serves to shuttle the complex out of the nucleus to the cytoplasm (fig. 3). This nuclear export mechanism is dependent on the activity of Crm-1/exportin 1 and is mediated through a carboxy-terminal leucine rich NES on IκB [62]. This shuttling mechanism can be blocked by the nuclear export inhibitor leptomycin B [61].

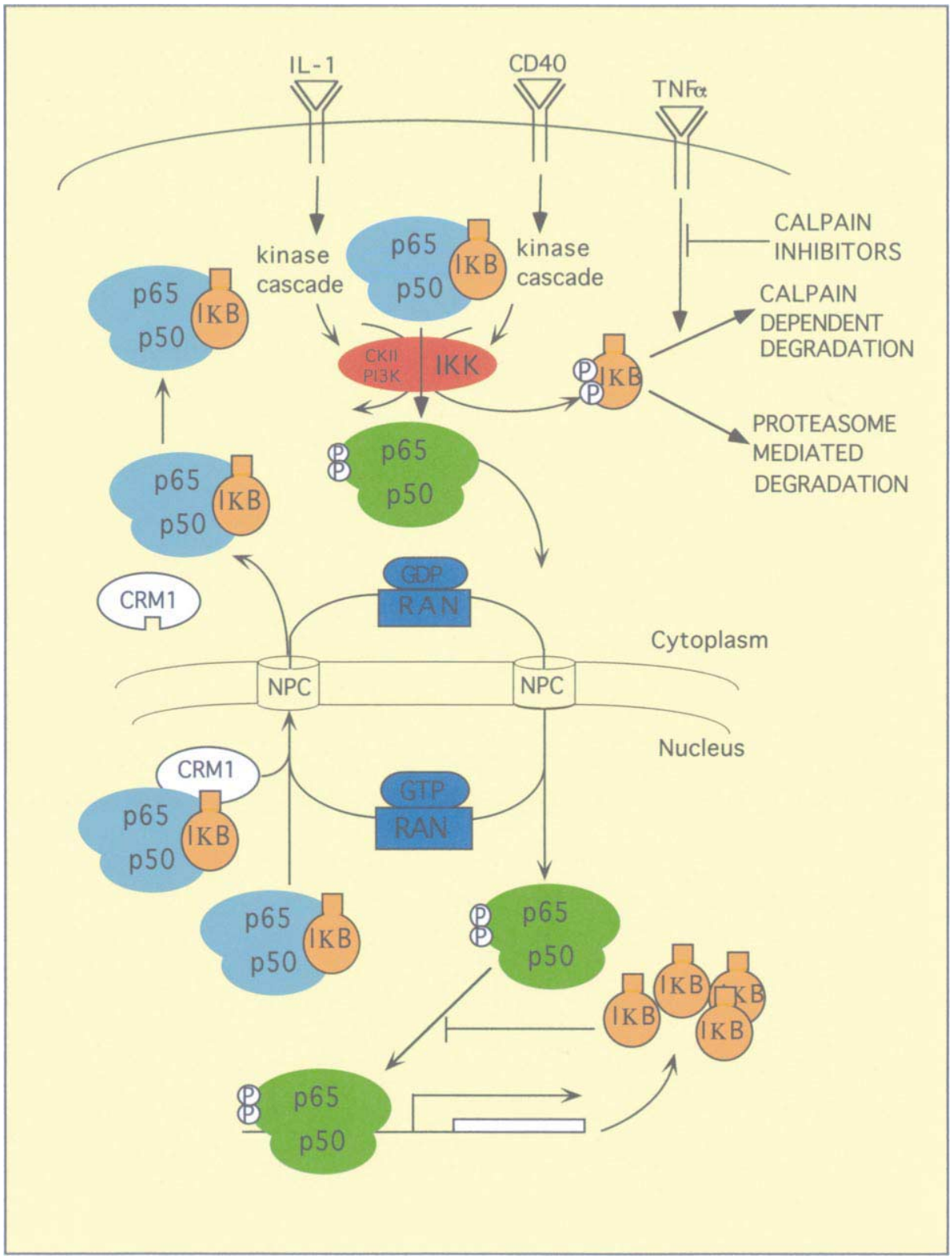

Figure 3. NFκB shuttling: Following cell surface receptor signaling, a kinase cascade is initiated that results in the phosphorylation and release of the IκB subunit from the NFκB dimer, which subsequently undergoes nuclear translocation. IκB is a direct gene target of NFκB, and hence a feedback loop is generated which functions to shut down NFκB activation by initiating nuclear export of the complex. The pathway is dependent on the Ran GTP/GDP cycle and on importins and exportins.

The mechanism of activation-inactivation of NFκB family members is probably the most well described mechanism of any transcription factor. Future work will most likely be focused on delineating the signals leading to IKK activation and hence the activation of NFκB.

p53

p53 is one of the most well described tumor suppressor proteins, found mutated in more than 50% of human tumors [64]. p53 is a transcription factor which is imported into the nucleus as a monomer [63]. Once nuclear, it forms homotetramers to generate an active DNA-binding transcriptional regulator. The major functional role of p53 is to induce cell cycle arrest or apoptosis in response to DNA damage to prevent damaged cells from dividing and duplicating damaged DNA in the daughter cells. The ability of p53 to cause cell cycle arrest or apoptosis is dependent on its ability to bind to specific DNA binding sites in the promoters of its target genes. p53-induced G1 arrest is associated with the transcriptional activation of the cyclin-dependent kinase inhibitor p21 [64, 65]. Another p53 target gene is *mdm2*, and its gene product Mdm2 (or the human homologue hdm2), binds directly to p53. Mdm2 possesses both an NLS [66] and NES [67], and accordingly it is constantly shuttling between the nucleus and the cytoplasm in a p53-independent fashion. Mdm2 therefore forms part of an autoregulatory feedback loop [68], whereby its production is controlled by p53 transcriptional activity, and by binding to p53 directly, it targets p53 for degradation in the cytoplasm.

A major mechanism for regulation of p53 function is that of cellular localization. As a transcription factor it requires nuclear import and retention in order to elicit its transcriptional and tumor suppressor activities. Moreover, it has been demonstrated that cytoplasmically sequestered p53 is incapable of tumor suppressor activity. In normal cells, p53 exists in very low levels in the nucleus in G1. This correlates with its predominant effect of inducing a G1 arrest in response to stress signals. The Mdm2 protein has been described as a mediator of the nuclear shuttling of p53 from the nucleus to the cytoplasm. Although Mdm2 has been ascribed the role of shuttling factor for p53, it has also been demonstrated that p53 itself can mediate its own nuclear export through an intrinsic leucine-rich NES located within its tetramerization domain [63]. This was examined using p53 mutants incapable of binding Mdm2. The formation of tetrameric p53 is believed to regulate Mdm2-independent nuclear export of p53. In the active tetrameric state, the NES in p53 is masked and inaccessible to export factors. This ensures that active p53 is maintained in the nucleus. However, when the p53 tetramer is dissolved and dimers or monomers are formed, the NES becomes accessible and the protein is efficiently exported from of the nucleus in a Crm1-dependent manner. In the more widely expressed model, Mdm2 regulates the transcriptional activity of p53 by binding to its transactivation domain, targeting p53 for nuclear export and subsequent degradation. Recently, it was demonstrated that Mdm2 can function as an E3 ligase specifically targeting p53 for ubiquitination and degradation [69]. Moreover, Mdm2 possesses a leucine-rich NES, and following p53 interaction this complex is removed from the nucleus in a manner dependent on Crm1/exportin 1 and on the Ran-GTP cycle [70]. The process of Mdm2-dependent degradation of p53 also requires an intact NES of Mdm2 [67, 70].

The tumor suppressor p19ARF was recently shown to be a regulator of p53 activity, and the growth-suppressive role of p19ARF is dependent on the presence of wild-type p53. p19ARF is localized in the nucleolus, and this localization is not dependent on the presence of p53 or Mdm2 [71]. p19ARF functions in the p53 pathway by binding to Mdm2 and sequestering it into the nucleolus, resulting in stabilized nuclear p53 [71]. An alternative proposal has been suggested whereby p14ARF (the human p19ARF homologue) binds Mdm2 and p53, sequestering the complex in the nucleus in the form of nuclear bodies which prevent export of p53 into the cytoplasm [72]. Following DNA damage, p53 becomes phosphorylated, which inhibits its affinity for Mdm2, hence stabilizing p53. The binding of p19ARF to Mdm2 reduces the Mdm2 ubiquitin ligase activity, also culminating in a stabilization of p53 [73].

A model can now be proposed for the regulation of p53 by subcellular localization (fig. 4). p53 can mediate its own nuclear/cytoplasmic shuttling via an intrinsic NES; however, this process may be enhanced by binding of Mdm2, acting as an E3 ligase to provide a more efficient or rapid export system. Alternatively, these shuttling pathways may represent two separate pathways converging with the same end result in the cytoplasm, that of proteasome-dependent p53 degradation. The mechanisms of sequestering p53 in the nucleus are not fully characterized, and some discrepancy exists as to the role of p19/p14 in this process. These and other mechanisms of p53 regulation at the nucleocytoplasmic transport level will need further investigation to be fully resolved.

NFAT family

The nuclear factor of activated T cells (NFAT) is a family of transcription factors (NFATc, NFATp, NFAT3, NFATx) that play a role in the transcriptional stimulation of cytokine genes such as IL-2, IL-4, CD40

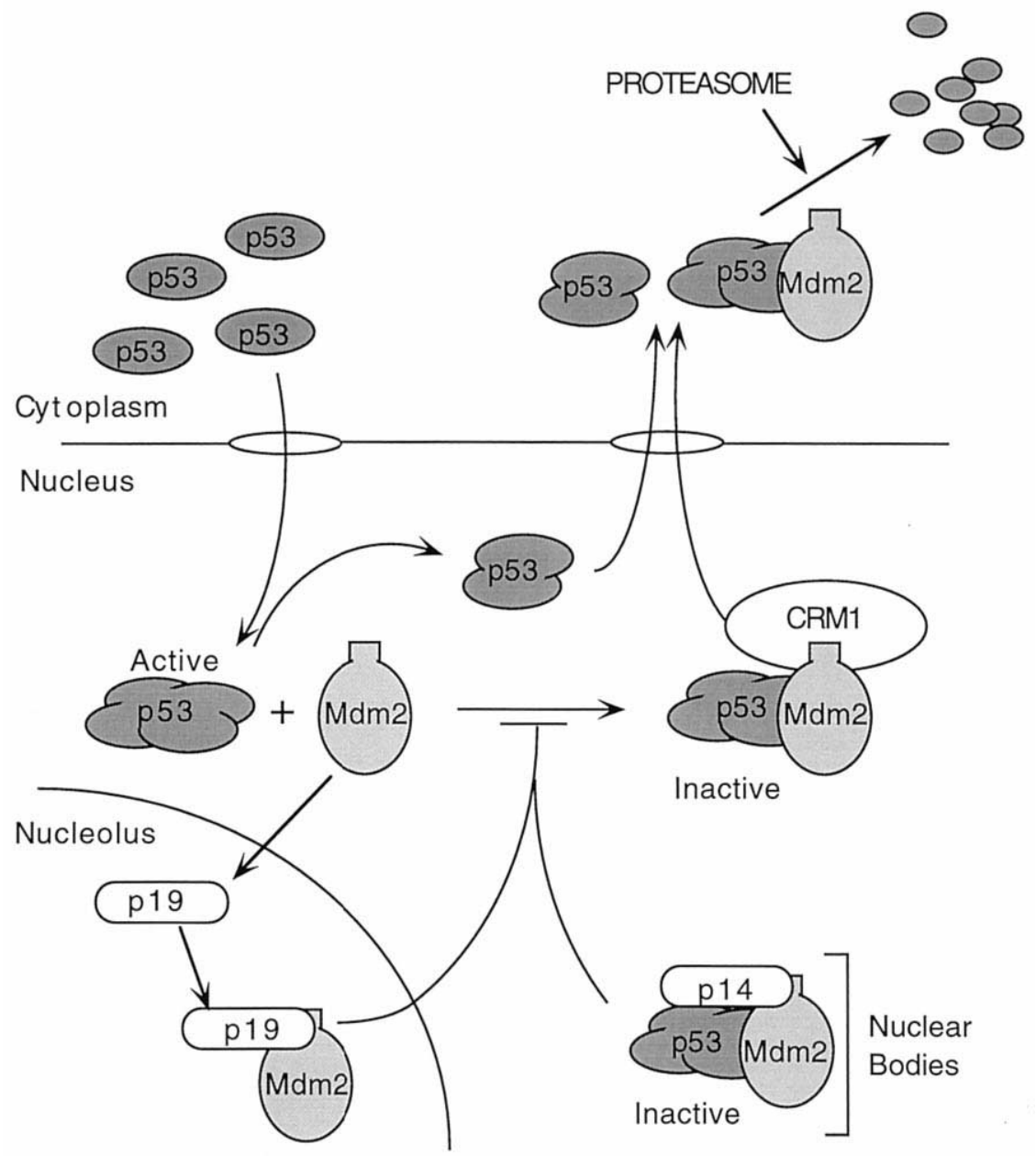

Figure 4. p53/Mdm2 shuttling: p53 is imported in to the nucleus in an importin-dependent manner to tetramerize to form the active DNA binding transcription factor. In the presence of Mdm2 its activity is inhibited, and the complex is either sequestered in the nucleus by p14/p19 or it is exported in a Crm1-dependent manner to undergo ubiquitination and proteasome-mediated degradation. See text for futher details.

ligand and Fas ligand [74]. They possess two regions of homology, a 300-residue NFAT homology domain and a DNA binding region that resembles the RHD of the REL family of transcription factors [75]. NFAT members form an active DNA binding complex with the AP-1 transcription component [76]. In resting cells these transcription factors are maintained in the cytoplasm in a highly phosphorylated state (fig. 5). In response to increased concentrations of calcium, a rapid nuclear import pathway is initiated, whereby NFAT is directly bound by the calcium-sensitive protein phosphatase calcineurin, and N-terminal dephosphorylation begins [77].

A specific calcineurin binding site (SPRIEIT) was identified in the amino-terminal region of NFAT, and mutations within this site prevented dephosphorylation of NFAT and hence blocked nuclear translocation [78]. The import process can be blocked by the calcineurin-specific drugs cyclosporin A or FK506 [79]. Binding of

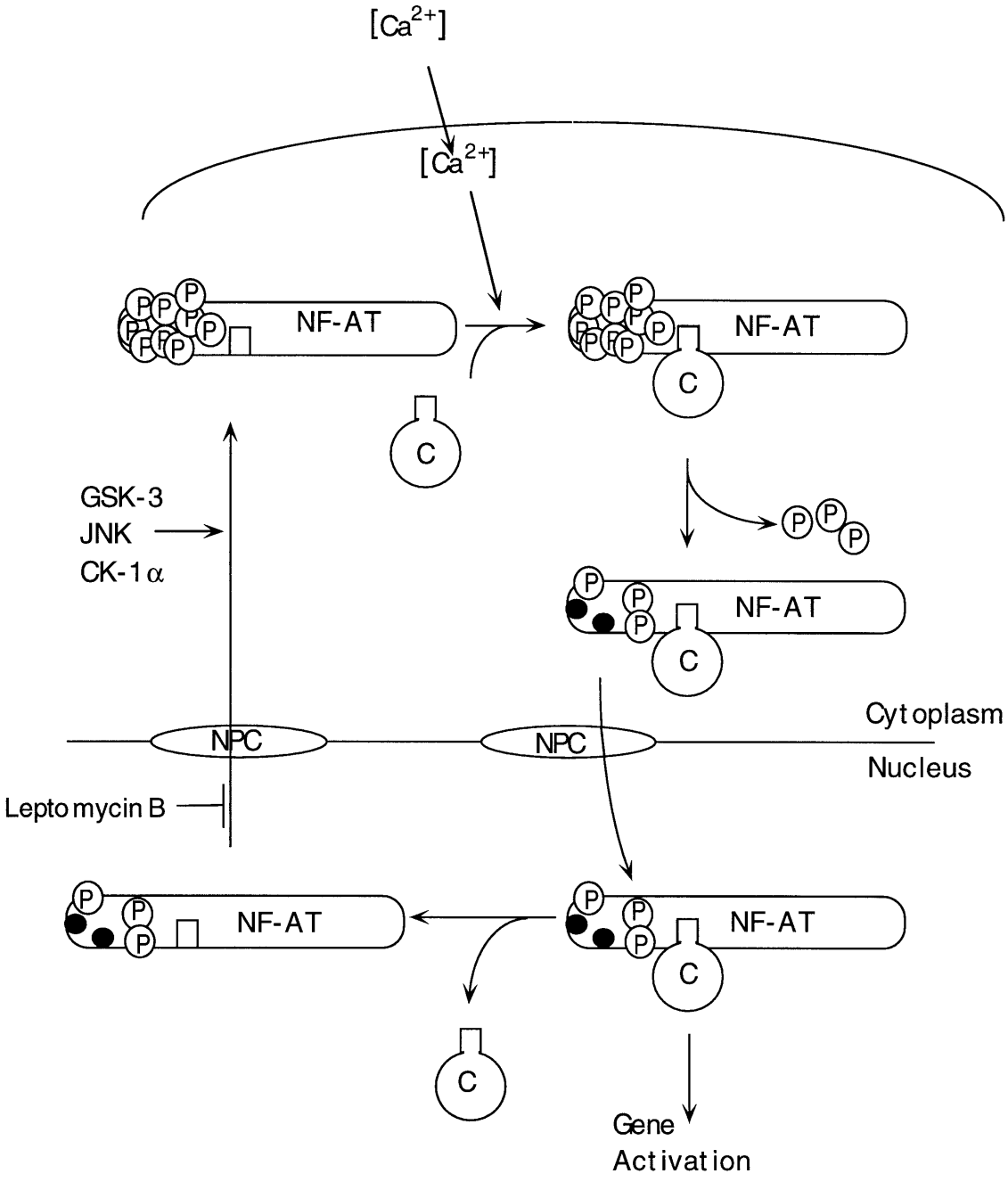

Figure 5. NFAT activation/inactivation: NFAT is maintained in the cytoplasm in a highly phosphorylated state. In response to increased levels of calcium, it is bound by the phosphatase calcineurin (C), and dephosphorylation is initiated. Dephosphorylation unmasks two NLS sequences (dark circles), and nuclear transport can take place in the presence of calcineurin, which masks the NFAT NES. When calcium concentrations are decreased, calcineurin binding is displaced to unmask an NES, and nuclear kinases initiate NFAT phosphorylation. The protein is rapidly exported, and cytoplasmic phosphorylation continues. The pathway involves the importins and exportins.

calcineurin to NFAT is maintained throughout nuclear translocation, and dephosphorylation of NFAT persists in the nucleus to act against a strong nuclear kinase activity [77]. The kinases responsible for phosphorylating NFAT have been identified as glycogen synthase kinase-3 (GSK-3) [80], JNK [81] and casein kinase 1α [82]. Phosphorylation of NFAT resulting from JNK activity promotes nuclear exclusion of NFAT [81]. Dephosphorylation results in the unmasking of two NLSs, leading to rapid nuclear accumulation (table 1) [83]. Binding of calcineurin to NFAT in the nucleus is essential for nuclear retention of the molecule [84]. Leptomycin B blocks the export, implicating Crm1 in the export pathway. Accordingly, it was demonstrated that binding of calcineurin to NFAT blocked the binding of Crm1 through the masking of an NES. The NFAT NES was initially identified by a rapamycin-inducible export assay [74] (table 1). Mutation of the NES was efficient in abolishing nuclear export of NFAT, providing evidence for its role as a bona fide NES. The end result achieved by the nuclear import/export shuttle of NFAT proteins is a very fast mechanism for regulation of the cellular response to calcium concentration.

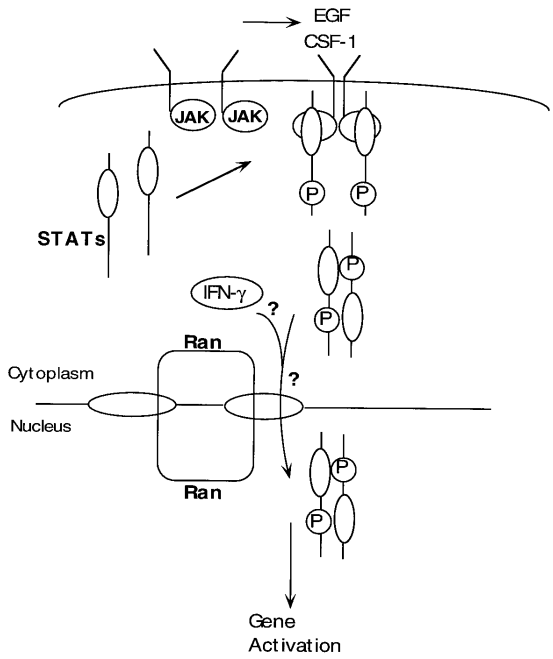

Figure 6. STAT activation: STATs are recruited to surface receptors following stimuli, where they become phosphorylated by the associated JAKs. Following phosphorylation, they undergo dimerization and are directed into the nucleus in a poorly defined mechanism. Once nuclear, they can mediate transcriptional activity. An involvement of the Ran GDP/GTP cycle has been proposed.

STATs

The STATs (signal transducers and activators of transcription) comprise seven members in mammals, and they are involved in eliciting transcriptional effects in response to cytokine signaling [85]. They were originally identified as downstream effectors in the IFN-γ signaling pathway [86]. All members share specific regions of homology, including a centrally located DNA binding domain, a SH3 domain with unspecified function, a carboxy-terminal transactivation domain, a highly conserved amino-terminal domain mediating protein-protein interactions, a SH2 dimerization domain and a carboxy terminally situated tyrosine residue important for mediating dimerization and nuclear localization [87]. This single tyrosine residue is the target of phosphorylation, which is absolutely required for STAT function [88]. This phosphorylated tyrosine residue is the target for the SH2 domain of an adjacent STAT member. The SH2 domain also functions as the domain responsible for STAT interaction with the surface receptors [85]. As monomers the STATs lack DNA binding activity and hence require dimerization in order to elicit their transactivation function [88, 89]. Not only are the STATs capable of dimer formation, the dimers can also promote the interaction with other dimers through the amino-terminal protein interaction domain to generate a cooperative DNA binding complex to allow for further diversity in promoter site recognition [90, 91]. The DNA recognition sequence of the STATs has been described, and it is apparent that although certain members share similar binding sites, they also possess individual specificity which greatly increases the diversity of potential target genes for STAT activation [90]. It has also been reported that different ligand-receptor signals can initiate different members of the family, which further increases the diversity of this family in terms of signaling [85]. This family of transcription factors has been the target of intense research recently, and recent reviews have discussed in detail the relative functions of each of the individual members [85, 92].

The mechanism of nuclear translocation of the STATs has been somewhat elusive. When inactive, the STATs are cytoplasmically localized, and upon receptor signaling by molecules such as EGF, PDGF or CSF-1, members of the Janus kinase (JAK) family are recruited to the receptor (fig. 6). The JAKs phosphorylate the cytoplasmic domains of the tyrosine kinase receptors, which in turn recruit STAT members to the receptor via their SH2 domains. Once the STATs are receptor associated, they are phosphorylated by the associated, JAKs on their essential tyrosine residue [85]. This phosphoryla-

tion initiates a pathway whereby homo- or heterodimerization of the STATs occurs in a head-to-tail fashion [89] (fig. 6). Following dimerization these complexes are translocated to the nucleus where they can activate transcription [89]. In this respect the STATs appear to be unique due to the fact that the activated molecule released from the cell surface receptors is the same molecule that translocates to the nucleus and binds DNA directly, implicating the absence of a cytoplasmic targeting cascade. The actual mechanisms of nuclear translocation have not been conclusively described, but there is emerging evidence that importins and the Ran-GTP/GDP cycle are mediators of the transport [93, 94]. STAT proteins lack an obvious NLS based on the conserved basic-rich sequence described earlier, which may indicate the involvement of non-STAT molecules in the process. Alternatively, a direct interaction of STAT members with the components of the NPC could account for this observation. In the case of IFN-γ signaling, it has been described that IFN-γ itself may mediate the nuclear import process of STAT1 through a basic-rich NLS located in its carboxy-terminal region [95]. In this case both IFN-γ and STAT1 are localized to the nucleus. The IFN-γ NLS was demonstrated to be capable of targeting a nonnuclear protein, allophycocyanin, to the nucleus [95], and hence this mechanism of piggyback translocation may provide a mechanism for STAT translocation. An alternative mechanism has been suggested based on results using STAT-ER (estrogen receptor) fusion constructs inducible with either estrogen or tamoxifen. In this model it is suggested that the dimerization of the STAT members is sufficient to unmask an intrinsic NLS within the STAT members, which would then mediate the nuclear translocation process independently of accessory factors [96]. Whether this mechanism is a general mechanism for all STAT members remains to be demonstrated.

Conclusions

In summary, we have reviewed what is known for the nuclear shuttling mechanisms of five transcription factor families. Examining these various mechanisms in detail, it is apparent that apart from their converging on the NPC in the form of an importin/exportin-bound complex mediated by the presence of NLS or NES sequence motifs, the signals leading to the initiation of their activation is not well conserved. Within each family, the members may be activated by different signals to generate an exquisite level of control over transcriptional activation and inactivation. Clearly the involvement of the importins and exportins, and the Ran-GTP/GDP cycle, are essential elements in regulating the transcriptional response following stimulation. Although rather well characterized, the mechanisms still possess several gaps which need to be filled for a complete understanding of how transcriptional control is regulated through cellular compartmentalization. For instance, the E2Fs have not been shown to require the importin and exportin complexes; however, this is likely since they contain NLS motifs. For p53, the mechanism of export is under intense investigation, and the binding partner Mdm2, has been proposed as the controlling factor for its export in combination with p19/p14. The mechanisms and signals leading to nuclear import of p53, however, have not been clearly defined. The main conclusion that can be drawn from analyzing these families is that many different mechanisms are required to elicit the correct transcriptional responses during the cell cycle, and each family has evolved its own specific mechanism for activation.

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