Journal of Cellular Biochemistry Supplements 32/33:32–40 (1999)

Dual Functions for Transcriptional Regulators: Myth or Reality?

René St-Arnaud

Genetics Unit, Shriners Hospital for Children, and Departments of Surgery and Human Genetics, McGill University, Montreal, Quebec, Canada

Abstract Several transcriptional regulatory molecules have been described that appear to possess dual function in separate cellular compartments. It remains unclear whether the proteins really exert dual functions, or which of the transcriptional regulatory role or the cytoplasm-associated function is the physiologically relevant action of the protein. This review will briefly describe the cases at hand and attempt to sort the true bifunctional proteins from the aritfactual trespassers. J. Cell. Biochem. Suppls. 32/33:32–40, 1999.

Key words: transcriptional control; coactivators

The precise control of gene transcription in particular tissues requires a delicate interplay between a wide variety of polypeptides. In addition to the components of the basic transcriptional machinery, tissue-specific gene transcription depends on the concerted actions of site-specific DNA-binding transcription factors, which in turn recruit a host of various cofactors that are essential for activated transcription. These cofactors have been ascribed different names, such as coactivators, mediators, or adaptors, but all appear to provide multiple protein-protein interfaces that allow to link the DNA-binding activators to the basic transcriptional machinery. The biochemical analysis of these coactivators revealed that some of them are not merely inert protein scaffolds but exhibit key enzymatic activities essential for transcriptional activation, such as phosphorylation or acetylation.

Some of the enzymatic functions and identified substrates for transcriptional coactivators are entirely consistent with their roles in transcriptional control. In recent years, however, several transcriptional regulatory molecules have been described that appear to possess dual function. These include the Sug1 and JAB1 coactivators which are components of the 26S proteasome; the PCD/DCoH cofactor that regulates dimerization of the HNF1 homeodomain protein but is also a cytoplasmic enzyme involved in tetrahydrobiopterin regeneration; β-catenin, which associates with cadherin in the cytoplasm to regulate cell adhesion and motility but gets translocated to the nucleus upon Wnt signaling to regulate transcription; and the αNAC coactivator, initially identified as a regulator of nascent polypeptide translocation across the endoplasmic reticulum (ER) membrane. In some cases, it remains unclear whether the proteins really exert dual functions, or which of the transcriptional regulatory role or the cytoplasm-associated function is the physiologically relevant action of the protein. This review briefly describes the cases at hand and attempts to sort the true bifunctional proteins from the artifactual trespassers.

SUG1 AND JAB1

A frequently used experimental strategy for identifying putative transcriptional coactivators involves the screening of complementary DNA (cDNA) libraries by the yeast two-hybrid protein-protein interaction assay. The cDNA encoding for proteins that interact with the activation domain of DNA-binding transcriptional activators are then tested in transient transfection assays for the potentiation of activator-dependent gene transcription. This strategy can lead to the identification of bona fide

Correspondence to: René St-Arnaud, Genetics Unit, Shriners Hospital for Children, 1529 Cedar Ave., Montreal, Quebec, Canada H3G 1A6.
E-mail: rst-arnaud@shriners.mcgill.ca

Received 3 September 1999; Accepted 7 September 1999

coactivators that potentiate gene expression by forming complexes at or near the transcriptional start site. It can be envisaged, however, that proteins that interact with activators to regulate subcellular localization, posttranslational modification, stability, or degradation would also be identified in such screens. If the co-transfected protein increases the stability of the activator, the net overall effect on gene expression can be identical to the effects observed when studying the function of a true coactivator.

Two examples dramatically illustrate this fact: mSug1 and JAB1. Mutations in the yeast SUG1 gene, encoding the putative ATPase Sug1, were initially identified in a screen for genes that rescued defects in the activation domain of the Gal4 transcription factor [Swaffield et al., 1992]. Early reports suggested that Sug1 interacted with the activation domains of Gal4 and VP16, as well as with TBP [Swaffield et al., 1995], the TATA-binding protein, a key component of the TFIID basal transcription factor. The murine Sug1 homologue, mSug1, was also cloned using the yeast two-hybrid system to identify transcriptional mediators that interact with nuclear hormone receptors [Lee et al., 1995], a large family of hormone-dependent transcription factors. Taken together, these observations supported a coactivator function for Sug1 proteins. A controversy arose when several highly related Sug1-like proteins (CAD proteins, for conserved ATPase domain) were identified as components of the 26S proteasome. The 26S proteasome is the macromolecular complex that mediates ATP- and ubiquitin-dependent extralysosomal degradation of the bulk of cellular proteins and degradation of short-lived proteins such as cell cycle regulators [Voges et al., 1999]. Until it was purified to homogeneity, the composition of the 26S proteasome remained uncertain, and several investigators claimed that Sug1 was a subunit of RNA polymerase II holoenzyme and that the 26S proteasome lacked Sug1 but contained a closely related protein with anti-Sug1 antibody cross-reactivity. The controversy was put to rest after the purification of the 26S proteasome by conventional chromatography in parallel with nickel-chelate affinity chromatography using a His_6-tagged subunit of the 20S core particle of the 26S proteasome [Rubin et al., 1996]. Sug1 always co-purified with the proteasome, whereas stoichiometric ratios of 0.05:1 were detected for the number of copies of Sug1 per holoenzyme. These results demonstrate that Sug1 is an integral component of the 26S proteasome and suggest that the effects of SUG1 mutations on transcription are indirect results of defective proteolysis. This hypothesis was confirmed for the murine homologue, mSug1, which was shown to regulate proteasome-mediated degradation of the vitamin D receptor [Masuyama and MacDonald, 1998].

The putative Jun-activation-domain-binding protein 1 (JAB1) coactivator was also identified using the yeast two-hybrid methodology in a screen that used the N-terminal activation domain of c-Jun as a bait [Claret et al., 1996]. The interaction of JAB1 with c-Jun stabilizes the binding of c-Jun homodimeric complexes on their cognate AP-1 DNA binding site, leading to potentiated transactivation. In these first assays, the subcellular localization and putative targets within the basic transcriptional machinery were not studied.

The JAB1 protein also surfaced when investigators attempted to identify new components of the 26S proteasome. In these studies, a new complex, distinct from the 26S proteasome, was eventually purified [Seeger et al., 1998]. This multiprotein complex, termed signalosome, consists of at least 8 subunits, including JAB1 and Trip15, the thyroid hormone receptor-interacting protein 15. The purified complex also contains the mammalian homologues of COP9 and COP11 and appears identical to the plant COP9 complex involved in light-mediated signal transduction [Wei et al., 1998]. Interestingly, the isolated JAB1-containing particle has kinase activity that phosphorylates the N-terminal activation domain of c-Jun on the key Ser63 and Ser73 residues, an essential step for c-Jun-mediated transactivation [Smeal et al., 1991]. Before the characterization of the signalosome, the c-Jun N-terminal kinases (JNKs) were the only previously identified kinases known to phosphorylate the transcription factor at the critical N-terminal activation domain. The non-phosphorylated c-Jun is ubiquitinylated and rapidly degraded in normally growing cells; it is thought that phosphorylation of c-Jun protects it from ubiquitinylation and prolongs its half-life.

An interesting model has been proposed for the involvement of the signalosome in c-Jun-mediated transcription. Through the JAB1 subunit, c-Jun would be recruited to the complex

and phosphorylated at its N-terminus, leading to enhanced stability of the c-Jun dimers with AP-1 sites and increased c-Jun-mediated transcription [Seeger et al., 1998]. The model is strengthened by the finding that the 55-kDa subunit of the complex, Sgn1, is the signal transduction repressor Gps1 (Gα pathway suppressor 1, the mammalian homologue of COP11). Overexpression of Gps1 suppresses JNK activity in vivo [Spain et al., 1996]. The specific c-Jun phosphorylation and the fact that at least three subunits of the signalosome carry mitogen-activated protein kinase kinase (MAPKK) activation loop motifs support a role for the particle in signal transduction [Seeger et al., 1998]. Additional support is provided by the homology with the plant COP9 complex that mediate the plant's responses to extracellular signals [Wei et al., 1998], and the observation that the signalosome can also phosphorylate IκBα, a regulatory subunit of the NFκB transcription factor [Seeger et al., 1998]. Taken together, these data suggest that the signalosome/COP9 complex is an important cellular regulator modulating multiple signaling pathways.

It is fairly obvious from these new studies that despite their capacity to modulate activator-dependent transcriptional responses, mSug1 and JAB1 act via mechanisms that are clearly distinct from the classical definition of transcriptional coactivators and should not be classified as such. The new results emphasize the link between the processes of protein degradation and transcriptional regulation as a means of regulating cellular responses, but caution about the premature conclusion that a protein interacting with activation domains and able to potentiate transcription is necessarily a transcriptional coactivator.

PCD/DCoH

Hepatic nuclear factor 1 (HNF1) is a DNA-binding transcription factor comprising an α-helical dimerization interface combined to an atypical homeodomain and a POU related domain. It is involved in transcriptional activation of many genes, including serum proteins such as albumin, or enzymes like alcohol dehydrogenase and phenylalanine hydroxylase [Lei and Kaufman, 1998]. Purification of HNF1 from liver demonstrated that it is associated with a small protein, DCoH (dimerization cofactor of HNF1) that stabilizes HNF1 homodimers [Mendel et al., 1991]. Two DCoH molecules bind to a dimer of HNF1, forming a transcriptionally active tetrameric complex. The DCoH protein does not bind DNA and does not activate transcription on its own.

In an apparently unrelated field, DCoH was identified as the enzyme pterin-4α-carbinolamine dehydratase (PCD; recently re-named PHS, for phenylalanine hydroxylase stimulator). PCD participates in the regeneration of tetrahydrobiopterin (Fig. 1), the cofactor for phenylalanine hydroxylase and other aromatic amino acid hydroxylases. The importance of PCD in the regeneration of tetrahydrobiopterin

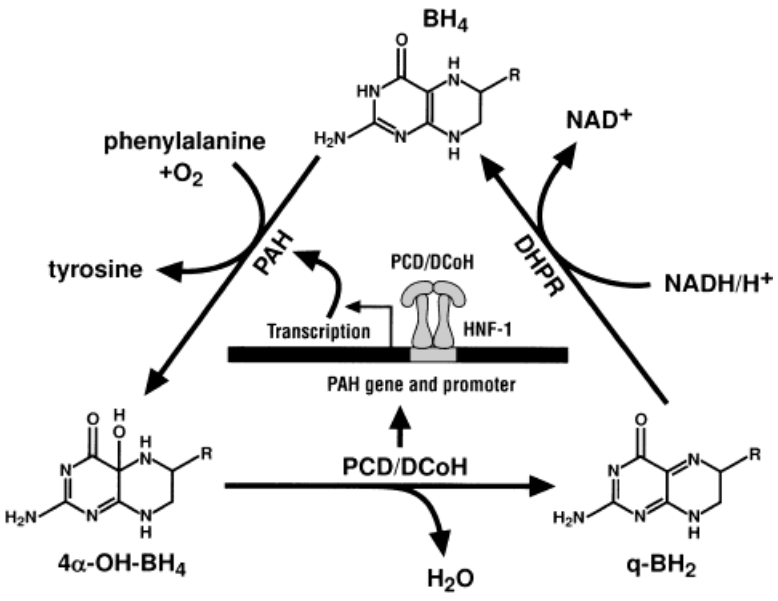

Fig. 1. Enzymatic and transcriptional regulatory functions of PCD/DCoH. (Periphery). During hydroxylation of aromatic amino acids such as tyrosine by phenylalanine hydroxylase (PAH), the cofactor tetrahydrobiopterin (BH_4; R = 1′,2′-dihydroxypropyl) is converted to 4α-hydroxytetrahydrobiopterin (4α-OH-BH_4). BH_4 is regenerated in two steps by PCD/DCoH and dihydropteridine reductase (DHPR). (Center) PCD/DCoH also stabilizes the HNF-1 dimer to help binding to the HNF-1 site within the PAH promoter, leading to increased PAH transcription.

is supported by the observations that certain hyperphenylalaninemic patients have mutations in this gene [reviewed in Suck and Ficner, 1996].

Is PCD/DCoH a true bifunctional molecule? How can the two apparently unrelated biological activities be reconciled? Recent results concerning the structure-function relationships of the protein and the analysis of putative HNF1 target genes shed new light on these puzzling questions.

The PCD/DCoH protein can be detected in both the cytoplasm and the nucleus [Sourdive et al., 1997], but there is no evidence that the phenylalanine hydroxylating system is present in the nucleus. PCD/DCoH is able to translocate to the nucleus on its own, and its subnuclear localization changes when HNF1 is present [Sourdive et al., 1997]. The crystal structure of PCD/DCoH shows that the protein is a tetramer containing two saddle-shaped grooves that have the potential to bind other macromolecules [Endrizzi et al., 1995]. The structure of PCD/DCoH is strikingly similar to that of TBP, the TATA binding protein, with β-sheets forming the concave part of a saddle structure, complete with "stirrups," and α-helices covering the convex side of the structure. Loops between the sheet and helices form the active center. Site-specific mutagenesis of PCD/DCoH has revealed that the enzymatic and transcriptional activity of the protein can clearly be dissociated [Johnen and Kaufman, 1997]. Two different aspects of the transcriptional role of PCD/DCoH were analyzed: interaction with HNF1 and ability to potentiate HNF1-dependent transcription. Interestingly, double mutations in the active center of the enzyme inhibit transcriptional potentiation but not HNF1 binding [Johnen and Kaufman, 1997]. The transcriptional function of PCD/DCoH therefore is composed of two parts—HNF1 binding and another contributing effect—that involve the active site and other molecular surfaces such as the saddle.

In this model, the coactivating function of PCD/DCoH is more consistent with a bridging function whereby it would act as an adapter between HNF1 and other components of the transcriptional apparatus. Such basal transcriptional machinery targets remain to be identified. It is interesting to note, however, that some mutations of PCD/DCoH, although neutral to enzymatic and HNF1-binding activities, increased the transcriptional potentiating activity [Johnen and Kaufman, 1997]. These results suggest that some of the macromolecular targets of PCD/DCoH could be transcriptional inhibitors. Because high levels of PCD/DCoH are required to prevent the formation of harmful isomeric forms of tetrahydrobiopterin, inhibitory modulation in the nucleus would provide a mechanism for maintaining subtle transcriptional regulation in the face of high concentrations of the factor.

One downstream target of HNF1 is the phenylalanine hydroxylase (PAH) gene [Lei and Kaufman, 1998]. HNF1 binding sites were identified within the human PAH promoter and PCD/DCoH was shown to potentiate HNF1-dependent PAH transcription [Lei and Kaufman, 1998]. These results support a dual function of PCD/DCoH in the context of the regulation of the phenylalanine hydroxylation system. Through its carbinolamine dehydratase activity, the enzyme regenerates the tetrahydrobiopterin cofactor that is essential for the hydroxylation of phenylalanine. In parallel, via its role in stabilizing and potentiating HNF1 dimers, it up-regulates the transcription of the PAH gene (Fig. 1).

This model elegantly reconciles the two functions of the molecule. To date, all the structure-function analysis remains in accord with the proposed dual roles. It will prove interesting to determine if similar use of the dual functions of PCD/DCoH can be identified for additional HNF1 transcriptional targets. Alternatively, HNF1 could recruit different partners in various context, although this possibility appears unlikely since protein-protein interaction screens failed to identify different dimerizing partners than PCD/DCoH [Sourdive et al., 1997]. The development of HNF1- PCD/DCoH cell-free transcription systems and a search for putative PCD/DCoH binding partners would provide novel tools to refine our understanding of the molecular mechanisms involved in the complex dual functions of this interesting protein.

β-CATENIN

Catenins were initially identified as proteins interacting with the cadherins, a large family of cell adhesion molecules that participate in cell-cell junctions [Aberle et al., 1996]. Three closely related family members have been identified: α-catenin, β-catenin, and γ-catenin (plakoglobin). The analysis of the Wnt/Wg signaling path-

way during development has led to the discovery that β-catenin plays key roles in adhesion-mediated signaling, from the cell membrane to the nucleus [Wodarz and Nusse, 1998].

β-catenin can be identified at three different locations inside the cell: at the cell membrane where it participates in adherens junctions with cadherins; in the cytoplasm, where a multiprotein complex regulates its stability; and in the nucleus, where it binds to transcription factors to regulate gene expression.

Cadherins bind catenins to form cytoskeletal complexes with the actin cytoskeleton that regulate cell-cell adhesion [Aberle et al., 1996]. Activation of several tyrosine kinases leads to phosphorylation of catenins and decreased cadherin-mediated adhesion. This signaling event appears to be reversible, as several protein tyrosine phosphatases have been shown to localize to sites of cell-cell contacts and interact with catenins, and could therefore counteract the effects of tyrosine phosphorylation of catenins.

In the cytoplasm, β-catenin is part of a complex that includes glycogen synthase kinase-3β (GSK3β), axin, and the adenomatous polyposis coli (APC) protein [Barth et al., 1997]. This complex regulates the stability of β-catenin. In the absence of signaling, GSK3β phosphorylates β-catenin and APC, thereby targeting β-catenin for ubiquitinylation and degradation by the proteasome. Multiple signaling events that include the interaction of Wnt/Wg molecules with the Frizzled receptors [Wodarz and Nusse, 1998] or the binding of extracellular matrix components to integrin receptors [Novak et al., 1998] lead to the inhibition of GSK3β activity, resulting in the stabilization of cytoplasmic β-catenin. Stabilization of β-catenin leads to its translocation to the nucleus via a nuclear localization signal (NLS)-independent pathway.

In the nucleus, β-catenin interacts with LEF/TCF factors (lymphoid enhancer factor/T-cell factor) to activate transcription. LEF/TCF proteins bind DNA through their high mobility group (HMG) DNA-binding domain [Eastman and Grosschedl, 1999]. Nuclear β-catenin may displace co-repressors of the Groucho family from DNA-bound LEF/TCF; because β-catenin contains two activation domains, the DNA-bound LEF/TCF-β-catenin dimer can then stimulate transcription.

The complexity of the Wnt signaling pathway and of the other pathways that regulate β-catenin function are beyond the scope of this article and the reader is referred to several recent reviews [Barth et al., 1997; Wodarz and Nusse, 1998; Eastman and Grosscheld, 1999]. What is important to note is that the cell uses β-catenin for two very distinct functions: cell-cell contacts and the regulation of gene transcription. Each function involves multiple protein-protein interactions. In *Xenopus* and *Drosophila*, Wnt/Wg signaling activates several genes involved in axis formation and segmental identity [Wodarz and Nusse, 1998]. The promoter of the mouse E-cadherin gene also contains a LEF/TCF binding site that recognizes the TCF/LEF-β-catenin complex. This finding suggests a link between the two roles of β-catenin: at the plasma membrane, β-catenin regulates cadherin-mediated adhesion by binding directly to cadherins; this regulation is enhanced by the regulation of cadherin gene expression by β-catenin in the nucleus.

αNAC

The Nascent Polypeptide Associated Complex (NAC) heterodimer was initially identified as a complex interacting with the nascent polypeptide chains as they emerge from the ribosome [Wiedmann et al., 1994]. The method used for identification and purification of NAC complexes involved ultraviolet (UV) cross-linking of proteins to ribosome-nascent chain complexes. Each polypeptide chain from the purified complex was subsequently identified: one subunit represented a novel polypeptide, termed αNAC, while the second protein had previously been identified and characterized as BTF3b [Wiedmann et al., 1994], a putative regulator of gene transcription [Parthun et al., 1992]. In the original model proposed for NAC function, the NAC dimer is essential for preventing signal sequence-independent binding of ribosomes to the translocation channel [Wiedmann et al., 1994]. Thus in the absence of NAC, all proteins would be incorrectly targeted to various subcellular compartments (Fig. 2).

Experiments addressing the physiological relevance of NAC in protein translocation appear technically challenging: the method used for ribosome extraction, the salt concentration of the buffers used, and the origin of the cell-free translation systems used appear to influence the outcome of the experiments. While the laboratory that initially identified NAC was able to conduct several series of experiments that con-

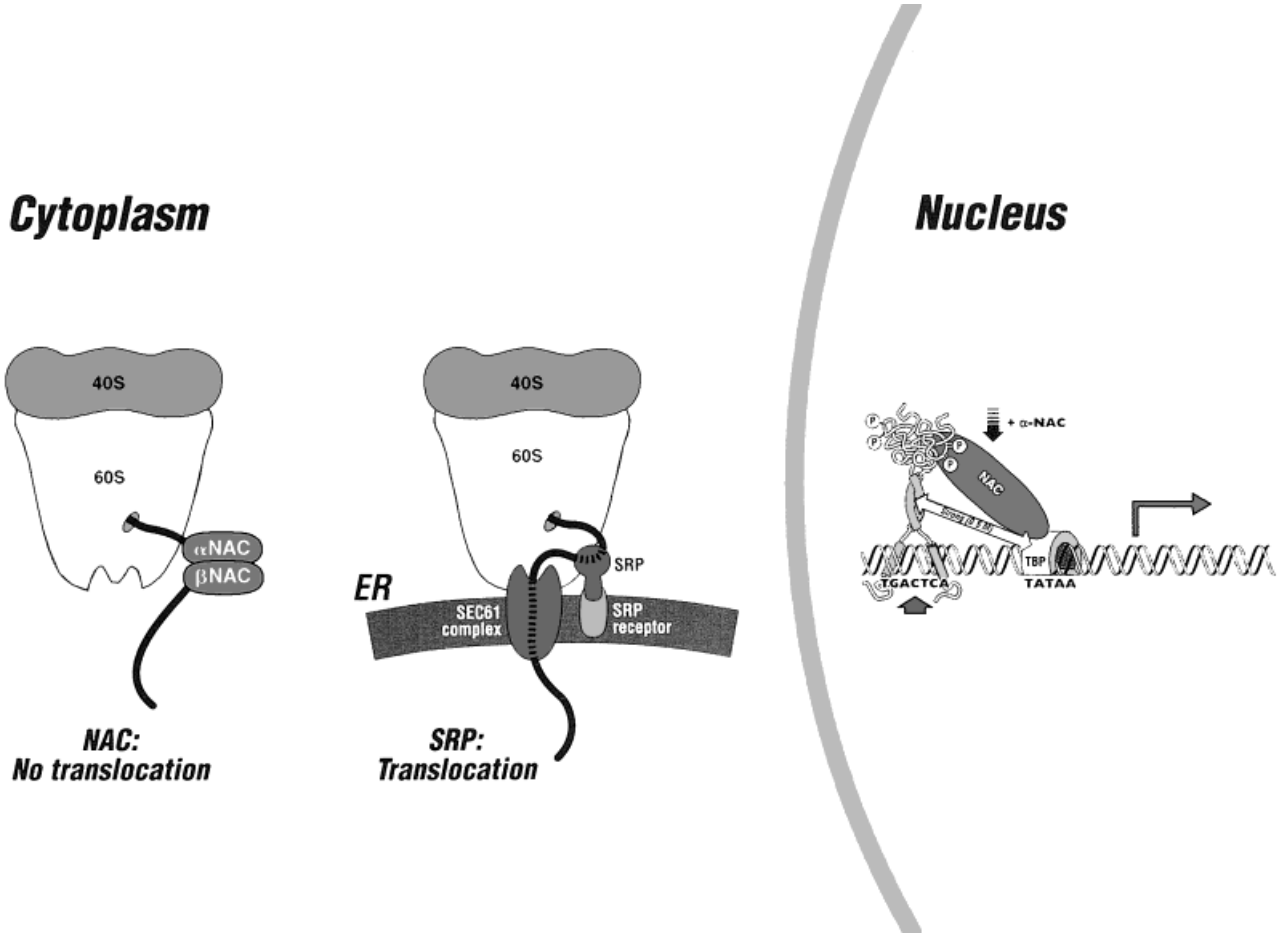

Fig. 2. Proposed roles of αNAC. In the cytoplasm, the NAC dimer binds the nascent polypeptides as they emerge from the ribosome, preventing incorrect translocation across the endoplasmic reticulum (ER) membrane. Nascent chains that possess a signal peptide are bound by the signal recognition particle (SRP), which efficiently targets these nascent chains to the ER, via interactions between SRP and its receptor. In the nucleus, αNAC potentiates c-Jun-mediated transcription. The αNAC protein interacts with the N-terminal domain of c-Jun. This interaction leads to a stabilization of the binding of the c-Jun homodimer with the AP-1 site. Because αNAC interacts strongly with both c-Jun and TBP, it strengthens the contacts between c-Jun and the basal transcriptional machinery, resulting in enhanced transcription from the c-Jun dimer.

firmed the role of the complex in the proper targeting of nascent chains across the translocation channel, efforts by other groups have not been successful and three different laboratories independently concluded that NAC does not prevent the binding of ribosomes to ER membranes and thus does not regulate translocation and sorting [Powers and Walter, 1996; Neuhof et al., 1998; Raden and Gilmore, 1998]. Recent results show that yeast mutants lacking αNAC are viable but suffer slight defects in the targeting of nascent polypeptides to several locations including the ER and mitochondria [George et al., 1998]. These new results appear to confirm some of the elements of the original model.

The difficulty in confirming the original model of NAC function is compounded by additional experimental results. It should not be forgotten that the dimerization partner of αNAC, BTF3b, was initially identified as a transcriptional regulator [Zheng et al., 1990]. Yeast strains deficient for the BTF3b homologue, egd1, show defects in the transcription of galactose-regulated genes [Parthun et al., 1992]. The mRNA for αNAC can only be detected past mid-gestation [Moreau et al., 1998], arguing against a key role for NAC in the regulation of protein translocation during development. The αNAC gene is also differentially spliced during development, and the muscle-specific form of NAC, termed skNAC (skeletal NAC), has been characterized as a transcription factor [Yotov and St-Arnaud, 1996]. Finally, a role for αNAC as a transcriptional coactivator has been described [Yotov et al., 1998; Moreau et al., 1998]. Taken together, these results strongly implicate αNAC in the regulation of gene transcription and raises the question as to whether the protein can truly exert dual functions.

We have identified αNAC in a screen for proteins specifically expressed in terminally differentiated osteoblasts. Antibodies raised against the recombinant αNAC protein allowed to demonstrate differential entry of the protein into the nucleus [Yotov et al., 1998]. These observations, coupled to the putative transcriptional regulatory role of the dimerizing partner BTF3b, prompted us to examine a putative role for αNAC in the control of gene transcription. We have shown that αNAC can interact with the GAL4/VP-16 chimeric activator, as well as with the c-Jun transcription factor, to potentiate their transcriptional activity [Yotov et al., 1998; Moreau et al., 1998]. We have also identified specific protein-protein interactions between αNAC and TBP, a component of the TFIID basal transcription factor [Yotov et al., 1998]. These types of interactions, which lead to potentiated transcription, define the coactivator class of proteins, allowing us to conclude that αNAC acts as a transcriptional coactivator.

An interesting feature of the NAC protein is that it can bind DNA at specific sites [Yotov and St-Arnaud, 1996]. It remains to be determined if the DNA-binding domain of αNAC is required for its coactivating function. A possible role for a specific DNA binding function within the αNAC molecule would be to target it to particular promoters, thus generating an increased level of specificity to the transcriptional response to αNAC during development. Interestingly, both the SV40 large T antigen and the myogenic transcription factor MEF2, which are sequence-specific DNA binding proteins, have been shown to act as coactivators without a requirement for their DNA-binding domain.

The subcellular localization of αNAC appears tightly regulated. This situation is reminiscent of the compartmentalization of β-catenin (see above) and could indicate that αNAC needs to be localized to distinct cell compartments to perform dual functions, as described for β-catenin. We have begun a careful structure-function analysis of the αNAC molecule and identified the domains that mediate nuclear localization of the protein. Identification of the signal transduction pathways that regulate αNAC localization and function will help to ascertain the physiological role of the protein.

Is αNAC a true bifunctional protein? Nuclear functions for ribosomal proteins have been described [Wool, 1996]. Because ribosomal biogenesis occurs in the nucleus, the regulation of transcription by "free" ribosomal proteins might be an important means of controlling gene expression by integrating transcription and translation. The difficulty that several laboratories have encountered in attempting to reproduce the original observation that NAC regulates the translocation of nascent polypeptides across the ER membranes raises questions about the physiological relevance of these observations. The structure of the αNAC gene and its differential splicing to generate a tissue-specific DNA-binding transcription factor, the nuclear localization and DNA-binding activity of αNAC, and the coactivating function that have been de-

scribed further argue against a key role for the protein in the regulation of the interactions of nascent polypeptides with the translocon. The αNAC-deficient yeast phenotype could be secondary to defects in gene transcription, as have been described for the βNAC yeast homologue [Parthun et al., 1996]. Additional experiments, including the engineering of αNAC-deficient cells and mice strains, will help elucidate the relevant function(s) of αNAC.

CONCLUDING REMARKS

Some of the examples presented here allow to conclude that some proteins can really function in a dual capacity. However, they also raise caution: it is not sufficient to detect an interaction between an activator and a cofactor leading to potentiation of gene transcription to proclaim that this cofactor is a true transcriptional coactivator. Indeed, indirect effects on gene transcription, such as those described for mSug1, can readily explain the transcriptional responses. The characterization of a particular gene product as a true transcriptional coactivator should include the identification of the protein target within the basic transcriptional machinery, an aspect that forms part of the definition of the coactivator class of molecules. This is not to say that any of the data outlined in this review were artifactual; it just underscores the fact that the regulation of gene transcription can happen via indirect mechanisms.

The recent experimental data discussed here allow us to draw the following conclusions: the interactions identified between transcription factors and mSug1 reveal the importance of the mechanisms controlling protein stability in the regulation of gene transcription. Similarly, the study of the JAB 1 protein permitted identification of a new complex that appears to integrate multiple signaling pathways. The evolutionary conservation of this complex supports its physiological importance.

There is no doubt that PCD/DCoH and β-catenin can carry multiple functions in cells. This suggests that the cell has selected means of increasing the efficacy of transcriptional control by combining functions. In the case of PCD/DCoH, this combination involves the synthesis of a key enzymatic cofactor coupled to a role in gene transcription, leading to a positive regulatory feedback loop. In the case of βcatenin, it is likely that inductive events initiated by signaling molecules during development necessitate the modulation of cell-cell interactions combined with increased transcription of target genes. β-catenin is ideally suited to integrate these events.

The jury is still out in the case of αNAC. Several pieces of information argue against the initial model proposed for NAC function; however, recent results from one laboratory continue to support a role for NAC in the regulation of the interactions between the ribosome and the translocon. Additional credibility for this model has recently been obtained through yeast genetics. Our own data on the function of αNAC, and the identification of the skNAC isoform, continue to point in a different direction. It is possible that the protein will really turn out to perform two distinct functions within cells, leading to reinforced pathways of gene expression through actions at different sites. There is no doubt that the entry of the αNAC protein into the nucleus is tightly regulated; this may have prevented several laboratories to identify the protein in the nuclear fraction. The careful structure-function analysis of the molecule, combined with the identification of the signal transduction pathways that control its subcellular localization, should help to understand the importance and the function of nuclear αNAC. Some of the mutants that have been engineered to analyze these aspects of αNAC function could then be tested for their effects on the interaction of NAC with ribosome-nascent chain complexes. We hope that the tools that are currently developed in several laboratories will soon allow to confirm the physiological role(s) of the NAC complex, and of αNAC in particular.

REFERENCES

Aberle H, Schwartz H, Kemler R. 1996. Cadherin-catenin complex: protein interactions and their implications for cadherin function. J Cell Biochem 61:514–523.

Barth AIM, Nathke IS, Nelson WJ. 1997. Cadherins, catenins and APC protein: interplay between cytoskeletal complexes and signaling pathways. Curr Opin Cell Biol 9:683–690.

Claret FX, Hibi M, Dhut S, Toda T, Karin M. 1996. A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. Nature 383:453–457.

Eastman Q, Grosschedl R. 1999. Regulation of LEF-1/TCF transcription factors by Wnt and other signals. Curr Opin Cell Biol 11:233–240.

Endrizzi JA, Cronk JD, Wang W, Crabtree GR, Alber T. 1995. Crystal structure of DCoH, a bifunctional, protein-binding transcriptional coactivator. Science 268:556–559.

George R, Beddoe T, Landl K, Lithgow T. 1998. The yeast nascent polypeptide-associated complex initiates protein targeting to mitochondria in vivo. Proc Natl Acad Sci USA 95:2296–2301.

Johnen G, Kaufman S. 1997. Studies on the enzymatic and transcriptional activity of the dimerization cofactor for hepatocyte nuclear factor 1. Proc Natl Acad Sci USA 94:13469–13474.

Lee JW, Ryan F, Swaffield JC, Johnston SA, Moore DD. 1995. Interaction of thyroid-hormone receptor with a conserved transcriptional mediator. Nature 374:91–94.

Lei X-D, Kaufman S. 1998. Identification of hepatic nuclear factor 1 binding sites in the 5′ flanking region of the human phenylalanine hydroxylase gene: implication of a dual function of phenylalanine hydroxylase stimulator in the phenylalanine hydroxylation system. Proc Natl Acad Sci USA 95:1500–1504.

Masuyama H, MacDonald PN. 1998. Proteasome-mediated degradation of the vitamin D receptor (VDR) and a putative role for SUG1 interaction with the AF-2 domain of VDR. J Cell Biochem 71:429–440.

Mendel DB, Khavari PA, Conley PB, Graves MK, Hansen LP, Admon A, Crabtree GR. 1991. Characterization of a cofactor that regulates dimerization of a mammalian homeodomain protein. Science 254:1762–1767.

Moreau A, Yotov WV, Glorieux FH, St-Arnaud, R. 1998. Bone-specific expression of the alpha chain of the nascent polypeptide-associated complex, a coactivator potentiating c-Jun-mediated transcription. Mol Cell Biol 18:1312–1321.

Neuhof A, Rolls MM, Jungnickel B, Kalies K-U, Rapoport TA. 1998. Binding of signal recognition particle gives ribosome/nascent chain complexes a competitive advantage in endoplasmic reticulum membrane interaction. Mol Biol Cell 9:103–115.

Novak A, Hsu S-C, Leung-Hagesteijn C, Radeva G, Papkoff J, Montesano R, Roskelley C, Grosschedl R, Dedhar S. 1998. Cell adhesion and the integrin-linked kinase regulate the LEF-1 and β-catenin signaling pathways. Proc Natl Acad Sci USA 95:4374–4379.

Parthun MR, Mangus DA, Jaehning JA. 1992. The EGD1 product, a yeast homolog of human BTF3, may be involved in GAL4 DNA binding. Mol Cell Biol 12:5683–5689.

Powers T, Walter P. 1996. The nascent polypeptide-associated complex modulates interactions between the signal recognition particle and the ribosome. Curr Biol 6:331–338.

Raden D, Gilmore R. 1998. Signal recognition particle-dependent targeting of ribosomes to the rough endoplasmic reticulum in the absence and presence of the nascent polypeptide-associated complex. Mol Biol Cell 8:117–130.

Rubin DM, Coux O, Wefes I, Hengartner C, Young RA, Goldberg AL, Finley D. 1996. Identification of the ga14 suppressor Sug1 as a subunit of the yeast 26S proteasome. Nature 379:655–657.

Seeger M, Kraft R, Ferrell K, Bech-Otschir D, Dumdey R, Schade R, Gordon C, Naumann M, Dubiel W. 1998. A novel protein complex involved in signal transduction possessing similarities to 26S proteasome subunits. FASEB J 12:469–478.

Smeal T, Binetruy B, Mercola DA, Birrer M, Karin M. 1991. Oncogenic and transcriptional cooperation with Ha-Ras requires phosphorylation of c-Jun on serines 63 and 73. Nature 354:494–496.

Sourdive DJD, Transy C, Garbay S, Yaniv M. 1997. The bifunctional DCOH protein binds to HNF1 independently of its 4α-carbinolamine dehydratase activity. Nucleic Acids Res 25:1476–1484.

Spain BH, Bowdish KS, Pacal AR, Staub SF, Koo D, Chang CY, Xie W, Colicelli J. 1996. Two human cDNAs, including a homolog of *Arabidopsis* FUS6 (COP11), suppress G-protein-and mitogen-activated protein kinase-mediated signal transduction in yeast and mammalian cells. Mol Cell Biol 16:6698–6706.

Suck D, Ficner R. 1996. Structure and function of PCD/DCoH, an enzyme with regulatory properties. FEBS Lett 389:35–39.

Swaffield JC, Bromberg JF, Johnston SA. 1992. Alterations in a yeast protein resembling HIV Tat-binding protein relieve requirement for an acidic activation domain in GAL4. Nature 357:698–700.

Swaffield JC, Melcher K, Johnston SA. 1995. A highly conserved ATPase protein as a mediator between acidic activation domains and the TATA-binding protein. Nature 374:88–91.

Voges D, Zwickl P, Baumeister W. 1999. The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu Rev Biochem 68:1015–1068.

Wei N, Tsuge T, Serino G, Dohmae N, Takio K, Matsui M, Deng X-W. 1998. The COP9 complex is conserved between plants and mammals and is related to the 26S proteasome regulatory complex. Curr Biol 8:919–922.

Wiedmann B, Sakai H, Davis TA, Wiedmann M. 1994. A protein complex required for signal-sequence-specific sorting and translocation. Nature 370:434–440.

Wodarz A, Nusse R. 1998. Mechanisms of WNT signaling in development. Annu Rev Cell Dev Biol 14:59–88.

Wool IG. 1996. Extraribosomal functions of ribosomal proteins. Trends Biochem Sci 21:164–165.

Yotov WV, Moreau A, St-Arnaud R. 1998. The alpha chain of the nascent polypeptide-associated complex functions as a transcriptional coactivator. Mol Cell Biol 18:1303–1311.

Yotov WV, St-Arnaud R. 1996. Differential splicing-in of a proline-rich exon converts αNAC into a muscle-specific transcription factor. Genes Dev 10:1763–1772.

Zheng XM, Black D, Chambon P, Egly JM. 1990. Sequencing and expression of complementary DNA for the general transcription factor BTF3. Nature 344:556–559.