Journal of Cellular Biochemistry 70:213–221 (1998)

Functional Subnuclear Partitioning of Transcription Factors

David Stenoien,[1] Z. Dave Sharp,[2] Carolyn L. Smith,[1] and Michael A. Mancini[1]*

[1]Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030
[2]University of Texas Institute of Biotechnology, San Antonio, Texas 78245

Abstract After many years of reductionistic approaches to characterize molecular mechanisms involved in transcription, the number of factors recognized to take part in this process has increased remarkably and continues to grow. When considering posttranslational modifications in conjunction with the large number of factors involved in modulating the activity of transcription complex components, the overall intricacy becomes staggering. After two decades of intensive molecular investigations, there has been a concerted effort to integrate these findings with cellular approaches to understand transcription on a more global level. This sort of reasoning actually revisits studies of approximately 20 years ago that considered the functional consequences of steroid receptor association with nuclear structure. With an abundance of new molecular probes and increasingly powerful instruments to detect them in fixed and, more recently, live cells, the issue of functional subnuclear organization is receiving increased attention. In this report, we focus on advances in characterizing the functional significance of transcription factor association with the nucleoskeleton. In particular, we consider recent biochemical and ''molecular morphology'' data that point to the importance of dynamic spatial and solubility partitioning of gene regulators with nuclear architecture. J. Cell. Biochem. 70:213–221, 1998.

Key words: transcription; nucleus; cell architecture; nuclear matrix

An important issue in any high-precision, high-efficiency transaction on DNA, in this case transcription, would seem to be local recruitment of the players to increase the probability of productive interactions. Do cells actually do this and, if so, how? For DNA binding proteins, there is the additional problem of locating and binding to the correct recognition sequence of target promoters. Have eukaryotic cells evolved a strategy to deal with this problem? The different filamentous components of the cytoskeleton are well appreciated for their role in organizing metabolic function in the cytoplasm, but the idea of a nuclear "skeleton" or "matrix" performing similar duties, although decades old [Berezney and Coffey, 1974], is not yet universally accepted. Noteworthy cytological advances have increased the appreciation of nuclear architecture, but biochemical identification of the fundamental structural components of the nucleoskeleton remains elusive. Perhaps similar to the increases in our understanding of cytoplasmic metabolism prior to the identification of cytoskeletal proteins, study of the link between nuclear organization and function continues with a significant focus on RNA synthesis and processing. Because early progress toward understanding nuclear structure–function issues arose from the study of transcription factor association with the nuclear matrix [Barrack et al., 1977], it is suitable that model systems now being developed are again using transcriptional regulators as the conduit to investigate subnuclear structure and function.

Clues to how transcription factors solve the problem of recognizing their specific target DNA sequences in a vast genome of nonspecific DNA surfaced years ago. Several groups noted that, after extraction with 1–2 M NaCl, there remained an insoluble framework consisting of RNA, protein, and ribonucleoproteins that was postulated to be involved in organizing nuclear

Contract grant sponsor: American Heart Association; Contract grant number: 9630033N; Contract grant sponsor: NIH; Contract grant number: DK38546; Contract grant sponsor: Department of Cell Biology, Baylor College of Medicine.

*Correspondence to: Michael A. Mancini, Ph.D., Department of Cell Biology, 117a Cullen Building, Baylor College of Medicine, Houston, TX 77030.
E-mail: mancini@bcm.tmc.edu

Received 27 February 1998; Accepted 27 February 1998

functions [Zbarski et al., 1962; Smetana et al., 1963; Steele and Busch, 1966; Narayan et al., 1967]. Based on the high content of RNA in this insoluble residue, it was proposed that these structures were involved in RNA processing and transport [Busch and Smetana, 1970]. Early attempts at isolating the nuclear framework left behind a complex mixture containing nuclear envelopes, nucleoli, ribonucleoprotein particles, RNA, and protein. Subsequent experiments were designed to isolate and identify the minimal components required to maintain the nuclear structure. Removal of phospholipids and soluble proteins by Triton X-100 extraction and cleaving of DNA by enzymatic digestions produced an nuclear matrix composed primarily of proteins and ribonucleoproteins [Berezney and Coffey, 1974, 1975, 1977; Berezney, 1980]. Development of several different techniques have improved the preservation and visualization of the underlying nuclear matrix [Capco et al., 1982, 1984; Fey et al., 1986; Jackson and Cook, 1988] and have demonstrated that the matrix consists of highly structured fibers that are interconnected with the nuclear lamina. This karyoskeleton, in fact, appeared to be part of a cellwide skeleton and was composed of a branched network of approximately 10-nm nuclear core filaments [Jackson and Cook, 1988; He et al., 1990; Nickerson et al., 1997]. It is important to note that, despite differences in the method used to isolate the nucleoskeleton (removing chromatin with physiological or high salt treatment or even after aldehyde prefixation), each protocol showed similar appearing filaments not unlike those found in the cytoskeleton. Nucleoskeleton skeptics nonplussed by wholemount or embeddment-free electron microscopic images that showed a cellwide filament system, including those within chromatin-depleted nuclei, were faced with evaluating two sets of filaments: the "familiar" intermediate filaments found in the cytoplasm and nuclear lamina and a morphologically similar set within the nucleus of unknown composition [Capco et al., 1982, 1984; Fey et al., 1986; Zhai et al., 1987; Jackson and Cook, 1988; He et al., 1990; Nickerson et al., 1997].

As procedures to isolate and characterize components of the nucleoskeleton evolved, evidence mounted that linked transcription with nuclear structure [Herman et al., 1978; Jackson et al., 1981, Ciejak et al., 1983; Robinson et al., 1982]. As mentioned above, several reports have demonstrated labeled steroid bound to components of nuclear structure [reviewed in Barack and Coffey, 1982; Alexander et al., 1987; Barrack, 1987]. These studies have suggested that, for a target tissue to respond to ligand, a major proportion of high-affinity and steroid-specific receptors would first associate with the nuclear matrix [Barrack, 1987]. Although these studies focused on specific, saturable steroid binding to target organ nuclei, the lack of immunological probes to these receptors hindered interpretation of these results. Despite the previous links between transcription, steroid receptors, and nuclear structure and perhaps due to the draw of newly acquired technical abilities in molecular cloning and continued difficulties in biochemically defining nucleoskeletal components, pursuit of additional matrix-bound regulators of transcription slowed. Although data linking actively transcribed genes with nuclear structure continued to appear [reviewed in Stein et al., 1995], few reports have specifically addressed transcription factors per se as bona fide residents of nuclear architecture.

Continued analyses to determine the composition of the nucleoskeleton led to several reports detailing both cell- and differentiation-specific changes in the two-dimensional gel profiles of nuclear matrix proteins (NMPs) [Capco et al., 1982; Fey and Penman, 1988; reviewed in Stein et al., 1995]. Indeed, a comprehensive and pivotal body of work concerning bone cell differentiation in vitro led to the identification of several NMPs that possessed specific, differentiation-dependent DNA binding activities [van Wijnen et al., 1993; Bidwell et al., 1993; Stein et al., 1995]. These studies were crucial for support of the notion that the nucleoskeleton could function to "concentrate" transcription factors [reviewed in Stein et al., 1995]. To advance the development of this paradigm, the NMPs responsible for the in vitro binding activities needed to be identified. Two NMPs, NMP-1 and NMP-2, were subsequently identified as the previously cloned transcription factors, YY1 and a AML family member, clearly substantiating the hypothesis that the nucleoskeleton could act as a repository for factors involved in transcription [Guo et al., 1995; Merriman et al., 1995]. These and other examples [Dworetzky et al., 1992; Bidwell et al., 1993; Sun et al., 1994; van Steensel et al., 1995; Tang and DeFranco, 1996; Mancini et al., 1994, 1998] complimented concepts brought to light in the

older steroid receptor-matrix data. Taken together with a stream of recent reports showing increased complexity of promoter–transcription factor interactions, it became decidedly less presumptive that the nucleoskeleton was somehow involved in the regulation of gene expression [Stein et al., 1995].

Both ligand- and differentiation-dependent interactions of regulator proteins with the nuclear matrix suggested that these functions were far from static. Additional interest in nuclear structure-influenced transcriptional regulation developed from the findings that the retinoblastoma protein (Rb) was shown to associate with nuclear structure in a cell-cycle-dependent manner [Mittnacht and Weinberg, 1991; Mancini et al., 1994]. Rb has been shown to interact with numerous transcription factors, both as a repressor and as an activator of transcription [Riley et al., 1994]. These reports shed new light on not only the concept of dynamic nuclear matrix associations but also demonstrate mechanistically that this association is regulated, in this case by phosphorylation. Perhaps most importantly, inactivating Rb mutations frequently found in several different tumors result in a complete loss of its ability to interact with nuclear structure [Mittnacht and Weinberg, 1991; Mancini et al., 1994]. A large number of Rb-interacting proteins have been identified [reviewed in Riley et al., 1994]. The observation that many Rb-binding proteins also interact with the nuclear matrix [Durfee et al., 1994; Mancini et al., 1994], including several inactivating viral oncoproteins, further suggests the importance of Rb–nuclear matrix interactions. One model of Rb function purports that a nuclear Rb "network" is critical for its tumor suppressor/cell cycle regulatory properties [Lee et al., 1994]. When speaking of nuclear proteins and cell cycle, one should also consider the reorganization during mitosis of structural proteins constituting the nuclear matrix. Indeed, Rb has been shown to interact with several "mitotic" proteins that have ties to nuclear structure during interphase. Futhermore, it has been shown that numerous nuclear matrix components contribute to the formation and function of the mitotic apparatus [He et al., 1995; Mancini, et al., 1996].

Based on the Rb data and observations that levels of several transcription factor activities derived from the nuclear matrix change during tissue-specific differentiation [van Wijnen et al., 1993], we became interested in developing a model system to examine the relationship between a known transcription factor and nuclear structure. We chose initially to study the pituitary-specific, POU-class transactivator Pit-1 for several reasons. The functional domains of Pit-1 have been well characterized, it is highly specific (only a few known target promoters, including growth hormone and prolactin), and immunological and molecular tools were readily available. Similar to other transcription factors [Grande et al., 1997], endogenous Pit-1 is spatially distributed in the nucleoplasm of cultured GH3 cells, a pituitary tumor cell line that secretes growth hormone and prolactin. Interestingly, the fine speckled pattern of Pit-1 was shown by laser scanning confocal microscopy to be reduced at SC-35 domains, intranuclear regions known to contain RNA splicing factors [Spector, 1993; Moen et al., 1995]. Several active genes have specifically been mapped to the periphery of SC-35 domains, suggesting a dynamic interplay between storage of splicing factors and transcription [Spector, 1993; Moen et al., 1995; Misteli et al., 1997]. Debate continues as to whether active genes are the cause or effect of this relationship [Singer and Green, 1997]. Investigations were designed to determine whether Pit-1 interacts with the nucleoskeleton and, if so, how. In situ and biochemical approaches showed that most of endogenous (or transfected) Pit-1 was extractable with detergents and that approximately 25% of Pit-1 partitioned with the nuclear matrix fraction [Mancini et al., 1995, 1998]. Extensive evaluation of deletion mutants and chimeric proteins showed that a distinct and highly conserved region of Pit-1, the 66-amino-acid pou-specific domain (PSD), was responsible for spatial and solubility partitioning in the nucleus [Mancini et al., 1995, 1998]. Another POU family member, Oct-1, was shown to partition similarly in both soluble and insoluble fractions [Kim et al., 1996], although the precise domain responsible for binding has not been critically studied. Although only a few proteins have been studied with the intent to determine residues necessary and sufficient for targeting the nucleoskeleton [Guo et al., 1995; van Steensel et al., 1995; Grondin et al., 1997; Zeng et al., 1997, 1998; Bushmeyer and Atchison, 1998; McNeil et al., 1998], it is clear that multiple sequence motifs are capable of this function. Along with the demonstration that the PSD can target heterolo-

gous proteins to the nucleoskeleton [Mancini et al., 1995, 1998], diverse targeting sequences for several other transcription factors have now been reported, including a 31-amino-acid sequence in the AML transcription factors [Guo et al., 1995] and an 84-amino-acid region of YY1 [Bushmeyer and Atchison, 1998; McNeil et al., 1998].

Throughout these types of investigations, a crucial question repeatedly surfaces: Where are the "functioning" transcription factors? Because a large body of evidence supports the idea that transcription is matrix associated, we concluded that the "functioning" pool of Pit-1 would be part of the nuclear-matrix-bound fraction. However, the detergent soluble fraction of Pit-1 is also known to function in site-specific DNA binding or in solid-phase transcription assays in vitro [Smith et al., 1995], as does Pit-1 derived from nuclear matrix preparations [Mancini and Sharp, unpublished observations]. Thus, an important partitioning distinction should be made with regard to functional versus functioning Pit-1 within the nucleus. Investigations designed to examine transcription factor interactions with repetitively integrated DNA binding sites [Htun et al., 1996; Robinett et al., 1996] in fixed or live cells will help in this regard. An additional advantage of the Pit-1 model is that several natural point mutations are known to cause pituitary dwarfism [Pfaffle et al., 1996]. Two of these inactivating point mutations were recapitulated in rat Pit-1 and then tested in subnuclear compartmentation assays in transfected CV1 and HeLa cells. Surprisingly, unlike the inactivating point mutations in Rb that resulted in a loss of nuclear matrix association, the A158P Pit-1 human dwarf and the W261C Pit-1 mouse dwarf mutations both partitioned completely with only the matrix fraction [Mancini et al., 1995, 1998]. These two natural mutations, one that retains Pit-1-specific DNA binding (A158) and the other that loses its DNA binding function, suggest the novel concept of a "partitioning mutation." Because wild-type Pit-1 routinely partitions to both detergent-extractable and nuclear-matrix-bound fractions, could it be that the ability of Pit-1 to function as an activator is influenced by its ability to interact with both compartments, perhaps to increase the likelihood of finding its relatively rare target promoters? This concept is supported in part by much older data with ligand-dependent binding of steroid receptors with the nuclear matrix [Barrack, 1987]. More recently, this notion has been advanced with the observation that glucocorticoid interaction with the nuclear matrix is ATP dependant [Tang and DeFranco, 1996].

Collectively, these disparate data support the idea that transcription factor partitioning with nuclear structure is codependent on movements "on and off" the transcription-competent nucleoskeleton. Additional support for this premise comes from subnuclear partitioning analyses with new inactivating Pit-1 mutations obtained in a novel yeast screen [Mancini et al., unpublished communications]. While retaining an intact activation domain in the N-terminus, a region containing the PSD and flanking amino acids was specifically mutagenized by using error-prone polymerase chain reaction and gap cloning in yeast. In each of the five new inactive Pit-1 mutants, partitioning dynamics were again completely skewed to the core filament fraction. Moreover, one mutation (Q95R) near the PSD was found to retain specific DNA binding activity in vitro but was functionally inactive in vivo. These data further support the idea that defects in subnuclear partitioning can negatively influence transcription factor function, even when an intact activation domain and specific DNA binding are unaffected.

With multiple lines of evidence to support a functional association between the transcription factors and the nucleoskeleton, characterization of the binding partners becomes a logical priority. A difficult hurdle hindering advances in this area is the obvious lack of available information regarding the composition of the nucleoskeleton. To date, the composition of the core filaments remains unknown, although two reports have suggested at least a partial involvement of several hnRNPs, lamin and NuMA [Mattren et al., 1997; Hozak et al., 1995; Zeng et al., 1994]. Of particular merit are several observations of regulatory factors interacting with matrix proteins. The hypophosphorylated, active form of Rb interacts with lamin A/C and a novel speckle domain protein, p84 [Durfee et al., 1994; Mancini et al., 1994]. In addition, a recent report has shown that the NMP hnRNP functionally interacts with the glucocorticoid receptor [Eggert et al., 1997]. Several low-molecular-weight matrix proteins have also been identified that interact with multiple steroid receptors [Lauber et al., 1995].

With the ability to identify sites of RNA synthesis using Br-UTP and/or antibodies to the hyperphosphorylated large subunit of RNA polymerase II (pol IIo) [Bregman et al., 1995; Mortillaro et al., 1996], it is now possible to visualize where nuclear regulators are in three-dimensional space relative to sites of new message synthesis. Reports of the number of pol II transcription sites per nucleus has ranged from several hundred to near 4,000 [Jackson et al., 1993; Wansink et al., 1993; Iborro et al., 1996; Fay et al., 1997]. Demonstration that a transcription factor would be found at a transcription site is, of course, not a surprise. Determination of the exact number of sites is perhaps much less exciting than the general principle of the observation that pol II transcription is not everywhere: it is confined spatially and by solubility. Although only a few transcription factors have been examined, it is clear that there is much less colocalization between RNA synthesis foci and transcription factors than expected. A quantitative, high-resolution immunofluorescence study of several transcription factors demonstrated that in whole cells labeled foci generally did not overlap with sites of transcription [Grande et al., 1997]. Does this lack of colocalization suggest a form of transcription regulation defined simply by spatial considerations? This observation is exemplified in Figure 1.

Immunofluorescent labeling of "active" pol II using the B3 monoclonal antibody [Mortillaro et al., 1996] shows a remarkably punctate pattern where the highest fluorescent signals falls off rapidly over a short distance. Moreover, a similar size, distribution, and number of foci are found in nucleoskeleton preparations [Stenoien et al., submitted]. Colocalization of pol IIo, in this case with a functionally active green fluorescent protein-tagged estrogen receptor, highlights the relatively small number of dual-labeled foci (Fig. 1). A recent colocalization study on matrix-bound transcription factors has also shown that most sites do not overlap with sites of transcription [Zeng et al., 1998; Stenoien et al., submitted]. These data raise the interesting possibility that transcription factors may not only shuttle the nucleoskeleton "on and off" [Barrack, 1987; Mancini et al., 1995, 1998; Lindenmuth et al., 1997] but also be able to "move" while bound. The matrix-bound foci that do not colocalize with sites of transcriptions may be in the process of recruiting initiation complex factors or, conversely, may have just ended a burst of transcription. With some transcription factors, such as steroid receptors, phosphorylation has been shown to contribute to activity. Thus, in addition to the above possibilities, it may be that posttranslational modifications to the receptor may influence its spatial relationship to sites of transcription. Development of immunological probes specific for this type of modification will be required to address this possibility.

The recent identification of a number of histone acetyltransferases and histone deacetylases has renewed interest in the role chromatin structure plays in the regulation of gene transcription [reviewed in Wolffe and Pruss, 1996; Pazin and Kadonaga 1997; Wu, 1997; Kadonaga, 1998]. Packaging genes into chromatin represses basal transcription, presumably because the DNA is tightly bound to the positively charged histone tails and is not accessible to transcription factors. Acetylation of lysine residues located within the amino terminal histone tails neutralizes their intrinsic positive charge, thereby reducing the affinity of DNA–histone interactions and facilitating access of transcription factors to the chromatin template [Wolffe, 1996]. In support of this hypothesis, core histone acetylation is generally correlated with increased transcriptional activity [reviewed in Pazin and Kadonaga, 1997], and treatment of cells with the histone deacetylase inhibitor trichostatin A increases progesterone receptor and retinoic acid receptor-mediated gene expression [Jenster et al., 1997; Minucci et al., 1997]. Conversely, core histone deacetylation is believed to repress gene expression [Pazin and Kadonaga, 1997]. In the last 3 years, a number of hormone-dependent transcriptional coactivators and corepressors for members of the steroid receptor superfamily have been identified [reviewed in Shibata et al., 1997]. Interestingly, several of the coactivators, such as SRC-1, CBP/p300, and the CBP-associated factor P/CAF, are histone acetyltransferases [Bannister and Kouzarides, 1996; Ogryzko et al., 1996; Yang et al., 1996; Spencer et al., 1997], and part of their ability to enhance target gene expression is likely derived from this enzymatic activity. In contrast, the corepressor proteins, N-CoR and SMRT, which in conjunction with unliganded retinoid and thyroid hormone receptors repress basal transcription and interact with the global repressor mSin3 and histone deacetylases [Heinzel et al., 1997; Nagy et al., 1997]. Thus, activation or repression of target

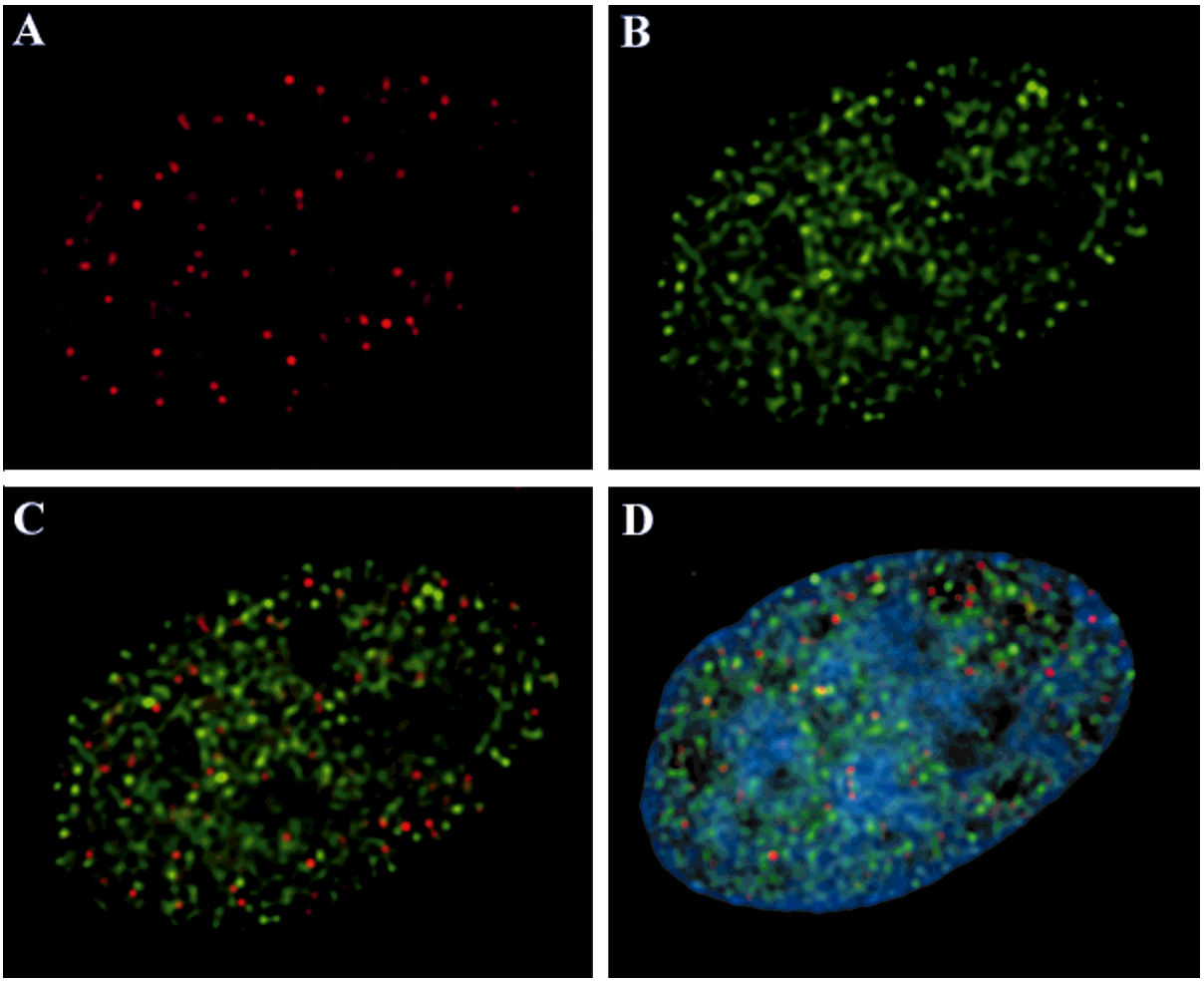

Fig. 1. Spatial relationship between a transcription factor and RNA polymerase IIo. Representative image of a whole HeLa cell transiently transfected with a transcriptionally active green fluorescent protein-tagged estrogen receptor (**B**; green) and immunolabed with an antibody to the hyperphosphorylated large subunit of RNA polymerase II (**A**; red) [Mortillaro et al., 1996]. A Z series of multiple focal planes were digitally imaged on a DeltaVision System with a highly sensitive CCD. The stack of images were deconvolved by a constrained iterative algorithm, creating the high-resolution optical sections shown in A–D (DeltaVision System, Applied Precision, Inc., Issaquah, WA). Only a small proportion of GFP-ER and polymerase IIo colocalize (**C**). In this study, cells were grown in serum containing medium. A merged image with DNA staining (DAPI) from a different focal plane is shown in **D**. Original magnification ×100.

gene expression by members of the steroid receptor superfamily appears to be mediated at least in part by histone acetyltransferase and/or deacetylase remodeling of chromatin structure. Interestingly, most histone acetyltransferase and deacetylase activity is associated with the nuclear matrix fraction [Hendzel et al., 1991, 1994; Davie, 1995], and this is consistent with observations from our laboratory that indicate that SRC-1 is a matrix-bound coactivator (data not shown). It remains to be determined whether steroid receptors recruit histone acetyltransferase and deacetylase activities to target gene promoters or if preformed coactivator/corepressor complexes enhance steroid receptor interactions with chromatin. With regard to the Rb story discussed above, several groups have shown that Rb associates with histone deacetylase [Luo et al., 1988; Brehm et al., 1998; Magnaghi-Jaulin et al., 1998]. Although probably multifactorial in nature, Rb interactions with deacetylases further suggest a functional relationship with nuclear architecture.

CONCLUDING REMARKS

The past two decades have brought an increased understanding of the mechanisms by which transcription is regulated. In vitro tran-

scription assays and transient transfections of transcription factors with reporter plasmids or, preferably perhaps, into cell lines with stably integrated reporters [Smith and Hager, 1997] have provided major insights into these mechanisms. Recent observations on the subnuclear partitioning of transcription factors and the role that chromatin remodeling plays in regulating transcription further support the notion that transcription needs to be studied in a more cellular context. Although the concepts have been long lived, we are just beginning to appreciate that gene transcription within the nucleus has multiple structural and functional dimensions and that nuclear architecture may play an important role in their establishment. Advances in imaging techniques and the next generation of probes to study the spatial distribution of transcription factors and the dynamics of transcriptional activation in both live and fixed cells will help increase our current level of understanding. An exciting area of future research will be to determine how all of the players involved in transcription are recruited to specific subnuclear domains and how their activities are coordinated to regulate transcription.

ACKNOWLEDGMENTS

We extend our appreciation to Maureen G. Mancini and Bing Liu for technical support that contributed to the primary data discussed in this report. This work was supported by a Scientist Development Grant (M.A.M.) from the American Heart Association (9630033N), NIH grant DK38546 (Z.D.S.), and the Department of Cell Biology, Baylor College of Medicine.

REFERENCES

Alexander RB, Greene GL, Barrack ER (1987): Estrogen receptors in the nuclear matrix: Direct demonstration using monoclonal antireceptor antibody. Endocrinology 120:1851–1857.

Bannister AJ, Kouzarides T (1996): The CBP co-activator is a histone acetyltransferase. Nature 384:641–643.

Barrack ER (1987): Steroid hormone receptor localization in the nuclear matrix: interaction with acceptor sites.

Barrack ER, Hawkins EF, Allen SL, Hicks LL, Coffey DS (1977): Biochem Biophys Res Commun 79:829–836.

Berezney R (1980): Fractionation of the nuclear matrix. I. Partial separation into matrix protein fibrils and a residual ribonucleoprotein fraction. J Cell Biol 85:641–650.

Berezney R, Coffey DS (1974): Identification of a nuclear protein matrix. Biochem Biophys Res Commun 60:1410–1417.

Berezney R, DS Coffey (1975): DS Nuclear protein matrix: Association with newly synthesized DNA. Science 189: 291–293.

Berezney R, Coffey DS (1977): Nuclear matrix. Isolation and characterization of a framework structure from rat liver nuclei. J Cell Biol 73:616–637.

Bidwell JP, Van Wijnen AJ, Fey EG, Dworetzky S, Penman S, Stein JL, Lian JB, Stein GS (1993): Osteocalcin gene promoter-binding factors are tissue-specific nuclear matrix components. Proc Natl Acad Sci USA 90:3162–3166.

Bregman DB, Du L, vanderZee S, Warren SL (1995): Transcription-dependent redistribution of the large subunit of RNA polymerase II to discrete nuclear domains. J Cell Biol 129:287–298.

Brehm A, Miska EA, McCance DJ, Reid JL, Bannister AJ, Kouzarides T (1998): Retinoblastoma protein recruits histone deacetylase to repress transcription. Nature 391: 597–601.

Busch H, Smetana K (1970): "The Nucleolus." New York: Academic Press.

Bushmeyer SM, Atchison ML (1998): Identification of YY1 sequences necessary for association with the nuclear matrix and for transcriptional repression functions J Cell Biochem 68:484–499.

Capco DG, Krochmalnic G, Penman S (1982): The nuclear matrix: Three-dimensional architecture and protein composition. Cell 29:847–858.

Capco DG, Krochmalnic G, Penman S (1984): A new method of preparing embeddment-free sections for transmission electron microscopy: Applications to the cytoskeletal framework and other three-dimensional networks. J Cell Biol 98:1878–1885.

Ciejek EM, Tsai MJ, O'Malley BW (1983): Actively transcribed genes are associated with the nuclear matrix. Nature 306:607–609.

Davie JR (1995): The nuclear matrix and the regulation of chromatin organization and function. Int Rev Cytol 162A: 191–250.

Durfee T, Mancini MA, Jones D, Elledge SJ, Lee WH (1994): The amino-terminal region of the retinoblastoma gene product binds a novel nuclear matrix protein that co-localizes to centers for RNA processing. J Cell Biol 127:609–622.

Dworetzky SI, Wright KL, Fey EG, Penman S, Lian JB, Stein JL, Stein GS (1992): Sequence-specific DNA-binding proteins are components of a nuclear matrix-attachment site. Proc Natl Acad Sci USA 89:4178–4182.

Eggert M, Michel J, Schneider S, Bornfleth H, Baniahmad A, Fackelmayer FO, Schmidt S, Renkawitz R (1997): The glucocorticoid receptor is associated with the RNA-binding nuclear matrix protein hnRNP U. J Biol Chem 272:28471–28478.

Fay FS, Taneja KL, Shenoy S, Lifshitz L, Singer RH (1997): Quantitative digital analysis of diffuse and concentrated nuclear distributions of nascent transcripts, SC35 and poly(A). Exp Cell Res 231:27–37.

Fey EG, Penman S (1988): Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc Natl Acad Sci USA 85:121–125.

Fey EG, Krochmalnic G, Penman S (1986): The nonchromatin substructures of the nucleus: The ribonucleoprotein (RNP)-containing and RNP-depleted matrices analyzed by sequential fractionation and resinless section electron microscopy. J Cell Biol 102:1654–1665.

Grande MA, van der Kraan I, de Jong L, van Driel R (1997): Nuclear distribution of transcription factors in relation to sites of transcription and RNA pol II. J Cell Sci 110: 1781–1791.

Grondin B, Cote F, Bazinet M, Vincent M, Aubry M (1997): Direct interaction of the KRAB/Cys2-His2 zinc finger protein ZNF74 with a hyperphosphorylated form of the RNA polymerase II largest subunit. J Biol Chem 44: 27877–27885.

Guo B, Odgren PR, van Wijnen AJ, Last TJ, Nickerson J, Penman S, Lian JB, Stein JL, Stein GS (1995): The nuclear matrix protein NMP-1 is the transcription factor YY1. Proc Natl Acad Sci USA 92:10526–10530.

He D, Zeng C, Brinkley BR (1995): Nuclear matrix proteins as structural and functional components of the mitotic apparatus. Int Rev Cytol 162B:1–74.

He D, Nickerson J, Penman S (1990): Core filaments of the nuclear matrix. J Cell Biol 110:569–580.

Heinzel T, Lavinsky RM, Mullen T-M, Soderstrom M, Laherty CD, Torchia J, Yang W-M, Brard G, Ngo SD, Davie JR, Seto E, Eisenman RN, Rose DW, Glass CK, Rosenfeld MG (1997): A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature 387:43–48.

Hendzel MJ, Delcuve GP, Davie JR (1991): Histone deacetylase is a component of the internal nuclear matrix. J Biol Chem 266:21936–21942.

Hendzel MJ, Sun JM, Chen HY, Rattner JB, Davie JR (1994): Histone acetyltransferase is associated with the nuclear matrix. J Biol Chem 269:22894–22901.

Herman R, Weymouth L, Penman S (1978): Heterogeneous nuclear RNA-protein fibers in chromatin-depleted nuclei. J Cell Biol 78:663–674.

Hozak P, Sasseville AM, Raymond Y, Cook PR (1995): Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. J Cell Sci 108:635–644.

Htun H, Barsony J, Renyi I, Gould DL, Hager GL (1996): Visualization of glucocorticoid receptor translocation and intranuclear organization in living cells with a green fluorescent protein chimera. Proc Natl Acad Sci USA 93:4845–4850.

Iborra FJ, Pombo A, Jackson DA, Cook PR (1996): Active RNA polymerases are localized within discrete transcription "factories" in human nuclei. J Cell Sci 109:1427–1436.

Jackson DA, Cook PR (1988): Visualization of a filamentous nucleoskeleton with a 23 nm axial repeat. EMBO J 7: 3667–3677.

Jackson DA, McCready SJ, Cook PR (1981): RNA is synthesized at the nuclear cage. Nature 292:552–555.

Jackson DA, Yuan, Cook (1988): A gentle method for preparing cyto- and nucleoskeletons and associated chromatin. J Cell Sci 90:365–378.

Jackson DA, Hassan AB, Errington RJ, Cook PR (1993): Visualization of focal sites of transcription within human nuclei. EMBO J 12:1059–1065.

Jenster G, Spencer TE, Burcin MM, Tsai SY, Tsai MJ, O'Malley BW (1997): Steroid receptor induction of gene transcription: A two step model. Proc Natl Acad Sci USA 94:7879–7884.

Kadonaga JT (1998): Eukaryotic transcription: An interlaced network of transcription factors and chromatin-modifying machines. Cell 92:307–313.

Kim MK, Lesoon-Wood LA, Weintraub BD, Chung JH (1996): A soluble transcription factor, Oct-1, is also found in the insoluble nuclear matrix and possesses silencing activity in its alanine-rich domain. Mol Cell Biol 16:4366–4377.

Lauber AH, Sandhu NP, Schuchard M, Subramaniam M, Spelsberg TC (1995): Nuclear matrix acceptor binding sites for steroid hormone receptors: A candidate nuclear matrix acceptor protein. Int Rev Cytol 162B:337–376.

Lee WH, Xu Y, Hong F, Durfee T, Mancini MA, Ueng Y, Chen PL, Riley D (1994): The corral hypothesis: A novel regulatory mode for retinoblastoma protein function. Cold Spring Harb Symp Quant Biol 59:97–107.

Lindenmuth DM, van Wijnen AJ, Hiebert S, Stein JL, Lian JB, Stein GS (1997): Subcellular partitioning of transcription factor during osteoblast differentiation: Developmental association of the AML/CBF/PEBP2-related transcription factor-NMP-2 with the nuclear matrix. J Cell Biochem 66:123–132.

Luo RX, Postigo AA, Dean DC (1998): Rb Interacts with histone deacetylase to repress transcription. Cell 92:463–473.

Magnaghi-Jaulin L, Groisman R, Naguibneva I, Robin P, Lorain S, Le Villain JP, Troalen F, Trouche D, Harel-Bellan A (1998): A retinoblastoma protein represses transcription by recruiting a histone deacetylase. Nature 391:601–605.

Mancini MA, Shan B, Nickerson JA, Penman S, Lee WH (1994): The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein. Proc Natl Acad Sci USA 91:418–422.

Mancini MA, Mancini MG, Liu B, Munoz-Medillin D, Sharp D (1995): J Cell Biochem 21B:140.

Mancini MG, Liu B, Sharp ZD, Mancini MA (1998): Subnuclear partitioning and functional regulation of the Pit-1 transcription factor. J Cell Biochem (in press).

Mattern KA, van Goethem RE, de Jong L, van Driel R (1997): Major internal nuclear matrix proteins are common to different human cell types. J Cell Biochem 65: 42–52.

McNeil S, Guo B, Stein JL, Lian JB, Bushmeyer S, Seto E, Atchison ML, Penman S, van Wijnen AJ, Stein GS (1998): Targeting of the YY1 transcription factor to the nucleolus and the nuclear matrix in situ: The C-terminus is a principal determinant for nuclear trafficking J Cell Biochem 68:500–510.

Merriman HL, van Wijnen AJ, Hiebert S, Bidwell JP, Fey E, Lian J, Stein J, Stein GS (1995): The tissue-specific nuclear matrix protein, NMP-2, is a member of the AML/CBF/PEBP2/runt domain transcription factor family: Interactions with the osteocalcin gene promoter. Biochemistry 34:13125–13132.

Minucci S, Horn V, Bhattacharyya N, Russanova V, Ogryzko VV, Gabriele L, Howard BG, Ozato K (1997): A histone deacetylase inhibitor potentiates retinoid receptor action in embryonal carcinoma cells. Proc Natl Acad Sci USA 94:11295–11300.

Misteli T, Caceres JF, Spector DL (1997): The dynamics of a pre-mRNA splicing factor in living cells. Nature 387:523–527.

Mittnacht S, Weinberg RA (1991): G_1/S phosphorylation of the retinoblastoma protein is associated with an altered affinity for the nuclear compartment. Cell 65:381–393.

Moen PT, Smith KP, Lawrence JB (1995): Compartmentalization of specific pre-mRNA metabolism: An emerging view. Hum Mol Genet 4:1779–1789.

Mortillaro MJ, Blencowe BJ, Wei X, Nakayasu H, Du L, Warren SL, Sharp PA, Berezney RA (1996): Hyperphosphorylated form of the large subunit of RNA polymerase II is associated with splicing complexes and the nuclear matrix. Proc Natl Acad Sci USA 93:8253–8257.

Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, Schreiber SL, Evans RM (1997): Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell 89:373–380.

Narayan KS, Steele WJ, Smetana K, Busch H (1967): Ultrastructural aspects of the ribonucleo-protein network in nuclei of Walker tumor and rat. Exp Cell Res 46:65–77.

Nickerson JA, Krockmalnic G, Wan KM, Penman S (1997): The nuclear matrix revealed by eluting chromatin from a cross-linked nucleus. Proc Natl Acad Sci 94:4446–4450.

Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y (1996): The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 87:953–959.

Onate SA, Tsai SY, Tsai MJ, O'Malley BW (1995): Science 270:1354–1357.

Pazin MJ, Kadonaga JT (1997): What's up and down with histone deacetylation and transcription? Cell 89:325–328.

Pfaffle R, Kim C, Otten B, Wit JM, Eiholzer U, Heimann G, Parks J (1996): Pit-1: Clinical aspects. Horm Res Suppl 1 45:25–28.

Riley DJ, Lee EY, Lee WH (1994): The retinoblastoma protein: More than a tumor suppressor. Annu Rev Cell Biol 10:1–29.

Robinett CC, Straight A, Li G, Willhelm C, Sudlow G, Murray A, Belmont AS (1996): In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. J Cell Biol 135:1685–1700.

Robinson SI, Nolkin BD, Vogelstein B (1982): The ovalbumin gene is associated with the nuclear matrix of chicken oviduct cells. Cell 28:99–106.

Shibata H, Spencer TE, Onate SA, Jenster G, Tsai SY, Tsai M-J, O'Malley BW (1997): Role of Co-activators and co-repressors in the mechanism of steroid–thyroid receptor action. Rec Prog Horm Res 52:141–165.

Singer RH, Green MR (1997): Compartmentalization of eukaryotic gene expression: Causes and effects. Cell 91: 291–294.

Smetana K, Steele WJ, Busch H (1963): A nuclear ribonucleoprotein network. Exp Cell Res 31:198–201.

Smith CL, Hager GL (1997): Transcriptional regulation of mammalian genes in vivo. A tale of two templates.

Smith KP, Liu B, Scott C, Sharp ZD (1995): Pit-1 exhibits a unique promoter spacing requirement for activation and synergism. J Biol Chem 270:4484–4491.

Spector DL (1993): Macromolecular domains within the cell nucleus. Annu Rev Cell Biol 9:265–315.

Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai M-J, O'Malley BW (1997): Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 389:194–198.

Steele WJ, Busch H (1966): Studies on the ribonucleic acid components of the nuclear ribonucleoprotein network. Biochim Biophys Acta 129:54–67.

Stein GS, van Wijnen AJ, Stein J, Lian JB, Montecino M (1995): Contributions of nuclear architecture to transcriptional control. Int Rev Cytol 162A:251–278.

Sun JM, Chen HY, Davie JR (1994): Nuclear factor 1 is a component of the nuclear matrix. J Cell Biochem 55:252–263.

Tang Y, DeFranco DB (1996): ATP-dependent release of glucocorticoid receptors from the nuclear matrix. Mol Cell Biol 16:1989–2001.

van Steensel B, Jenster G, Damm K, Brinkmann AO, van Driel R (1995): Domains of the human androgen receptor and glucocorticoid receptor involved in binding to the nuclear matrix. J Cell Biochem 57:465–478.

van Wijnen AJ, Bidwell JP, Fey EG, Penman S, Lian JB, Stein JL, Stein GS (1993): Nuclear matrix association of multiple sequence-specific DNA binding activities related to SP-1, ATF, CCAAT, C/EBP, OCT-1, and AP-1. Biochemistry 32:8397–8402.

Wansink DG, Schul W, van der Kraan I, van Steensel B, van Driel R, de Jong L (1993): Fluorescent labeling of nascent RNA reveals transcription by RNA polymerase II in domains scattered throughout the nucleus. J Cell Biol 122:283–293.

Wolffe AP (1996): Histone deacetylase: A regulator of transcription. Science 272:371–372.

Wolffe AP, Pruss D (1996): Targeting chromatin disruption: Transcription regulators that acetylate histones. Cell 84:817–819.

Wu C (1997): Chromatin remodeling and the control of gene expression. J Biol Chem 272:28171–28174.

Yang X-J, Ogryzko VV, Nishikawa J, Howard BH, Nakatani Y (1996): A p300/CBP associated factor that competes with the adenoviral oncoprotein E1A. Nature 382:319–324.

Zbarski IB, Dmitriev NP, Yermolayeva LP (1962): On the structure of tumor cell nuclei. Exp Cell Res 27:573–576.

Zeng C, He D, Brinkley BR (1994): Localization of NuMA protein isoforms in the nuclear matrix of mammalian cells. Cell Motil Cytoskel 29:167–176.

Zeng C, van Wijnen AJ, Stein JL, Meyers S, Sun W, Shopland L, Lawrence JB, Penman S, Lian JB, Stein GS, Hiebert SW (1997): Identification of a nuclear matrix targeting signal in the leukemia and bone-related AML/CBF-alpha transcription factors. PNAS 94:6746–6751.

Zeng C, McNeil S, Pockwinse S, Nickerson JA, Shopland L, Lawrence JB, Penman S, Hiebert S, Lian JB, van Wijnen AJ, Stein JL, Stein GS (1998): Intranuclear targeting of AML/CBF regulatory factors to nuclear matrix-associated transcriptional domains PNAS 95:1585–1589.

Zhai ZH, Nickerson JA, Krochmalnic G, Penman SJ (1987): Alterations in nuclear matrix structure after adenovirus infection. Virology 61:1007–101.