[Frontiers in Bioscience 8, d360-368, January 1, 2003]

TRANSCRIPTIONAL REGULATION OF THE APOLIPOPROTEIN AI GENE

Sohail Malik

Laboratory of Biochemistry and Molecular Biology, Rockefeller University, 1230 York Ave, New York, NY 10021

TABLE OF CONTENTS

1. ABSTRACT

Since the original description of the gene encoding apolipoprotein AI (apoAI) considerable progress has been made in identifying the regulatory regions and corresponding transcription factors that regulate its transcription in liver and intestinal cells. Nuclear receptors (in particular HNF-4) have emerged as the dominant (although by no means the exclusive) transcriptional activators that drive high levels of apoAI gene expression. In this review, some of the mechanisms (including interactions with recently described coactivators, as well as synergism with additional transcription factors bound to proximal and distal enhancers) that may underlie this process are discussed. Furthermore, apoAI gene expression has long been known to be subject to a variety of developmental, hormonal, dietary and pharmacological stimuli. The review thus also describes selected examples of these phenomena with the aim of highlighting how the operative transcriptional mechanisms are likely to be continually modified while maintaining the appropriate output.

2. INTRODUCTION

Apolipoprotein AI (apoAI) is the major protein component of plasma high density lipoprotein (HDL) (1-3). Consistent with epidemiological and genetic studies (including direct analyses of transgenic animals (4)), as well as many of its physical-chemical characteristics, plasma HDL levels of apoAI are inversely correlated with atherosclerosis (3). A primary role for apoAI is to promote cholesterol and phospholipid efflux from peripheral tissues as part of reverse cholesterol transport to the liver (3). Other aspects of apoAI physiology, such as a potential anti-inflammatory role (5), also may contribute to its overall cardioprotective effects.

The gene encoding mammalian apoAI is predominantly expressed in the liver and intestine. Although this gene is expressed at constitutively high levels that result in serum concentrations of apoAI of up to 14 mg/ml, it is subject to regulation (at the level of transcription) in response to a variety of pharmacological, dietary and physiological stimuli (3) (discussed further below). Given the critical importance of apoAI to human health and disease, and the associated need to understand how it is controlled at the transcriptional level, substantial progress has been made in describing the phenomenological aspects of this process. The present review summarizes these developments and attempts to put them in a broader perspective relative to other major advances in understanding transcriptional regulation in general. It summarizes various protein-DNA and protein-protein interactions that have been described to date as being responsible for apoAI gene expression and, importantly, emphasizes the dynamic nature of these interactions. The focus is primarily on apoAI expression in liver cells as corresponding analyses in intestinal cells are lagging. Furthermore, in as much as the recent discovery of novel transcriptional cofactors (6-8) has ushered in a new phase of investigation into model gene systems, the review also identifies future areas of research that might be useful toward obtaining a more complete view of the transcriptional controls prevailing at the apoAI gene.

3. GENERAL TRANSCRIPTION MECHANISMS

Current models view tissue-specific and developmental expression of RNA polymerase II (Pol II)-transcribed protein-encoding genes in eukaryotes as occurring via multiprotein complexes assembled on enhancer and core promoter sequences (9). In general, the

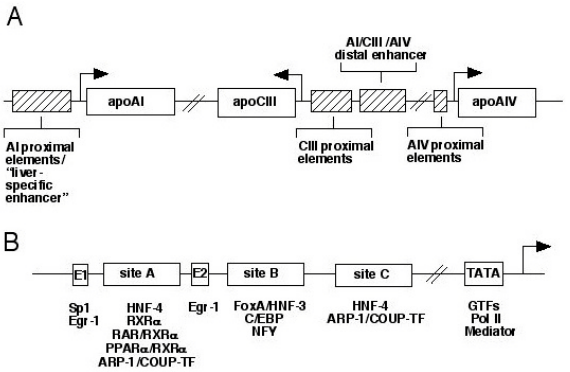

Figure 1. Organization of the apoAI gene. A: Schematic drawing showing the relative location of the apoAI gene relative to others in the cluster. Cross-hatched boxes indicate the identified regions, which contain binding sites for distinct transcription factors, both promoter-proximal and promoter-distal, that control individual genes in the cluster either on their own, or in synergy with each other. The locations and sizes of individual elements are not to scale. Arrows mark the relative position and orientation of the transcription start sites. B: Blow-up of the apoAI gene liver-specific enhancer (also indicated in panel A) to show some of the salient transcription factor binding sites (A,B, C, and E1/E2). Some of the interacting factors are shown. TATA indicates the TATA box, which together with the initiation site (arrow), nucleates assembly of the preinitiation complex containing Pol II, general transcription factors (GTFs) and Mediator.

factors assembled at these sites represent two distinct classes of transcription factors. Core promoter sequences (consisting of various combinations of the TATA box, initiator (Inr) and downstream proximal element (DPE)) (10) are recognized by Pol II-associated general transcription factors (GTFs). These include TFIIA, TFIIB, TFIID (which consists of the TATA box binding protein, TBP, and its associated factors, TAF_{II}s), TFIIE, TFIIF, and TFIIH (11, 12). These GTFs suffice for low (basal) level transcription of essentially all genes, at least in vitro. The second class of factors that typically bind to upstream regulatory elements, or enhancer regions, display a much greater degree of gene- and cell-type-dependent variability (13). Indeed multiple factors of this second class may regulate a given gene and impart on it a unique spatial and temporal transcription program. (Note that these factors include both activators and repressors but the emphasis here will be on the former.)

Coactivators, members of an additional class of transcription factors, have recently emerged as critical players in the process that translates the regulatory potential of enhancer-bound factors into enhanced activity of Pol II (6-8). In the broadest terms, two groups of coactivators can be described. The first includes TRAP/SMCC/Mediator (hereafter referred to as Mediator), a recently described multiprotein complex that is evolutionarily related to the yeast SRB/Mediator complex (14, 15) and is required for transcription from both DNA as well as more-physiological chromatin templates (6, 7). Mediator is a multisubunit complex, each of whose up to 25 constituent polypeptides potentially can serve as a target for different transcriptional activators. Indeed, since it appears that multiple activators can simultaneously target Mediator (e.g. the thyroid hormone receptor, TR, through the TRAP220 subunit and the tumor suppressor protein, p53, through TRAP80), it has also been proposed that this may contribute toward the well-established phenomenon of transcriptional synergism (7). Mediator has thus emerged as a central factor in transcription control.

Another group of coactivators, which include the various ATP-dependent chromatin remodeling complexes (e.g. Swi/SNF (6)) as well as the histone acetyl tranferase (HAT) activities (e.g. CBP/p300 and SRC-1 (16)) function primarily from chromatin templates. More recently, histone methylases also have been implicated in regulating gene activity at this level (17). As discussed in further detail below, a favored general model views the activator- and coactivator-dependent transcriptional activation as a sequential two-step process (7, 8, 18). In the first step, chromatin coactivators, recruited by the enhancer-bound transcription factors, may facilitate initial penetration of the barrier imposed by chromatin. Subsequently, Mediator, which also is recruited by the activators, could facilitate Pol II and GTF recruitment leading to activated levels of transcription.

4. ORGANIZATION OF THE APOAI GENE AND TRANSCRIPTION FACTORS INVOLVED IN ITS TRANSCRIPTION

4.1. Proximal and distal regulatory elements that direct apoAI gene expression

The apoAI gene is located within the genetically and physically linked cluster that also contains the genes encoding apoCIII and apoAIV, two other lipoproteins (19, 20) (Figure 1). Each of the genes of the cluster contains regulatory elements for transcription factors that drive the expression of the individual genes. At the same time, the genes share regulatory features, which ensure some degree of tandem control (21-23) (Figure 1A).

Multiple approaches (including deletion mapping of the regulatory regions of the apoAI gene in cultured hepatocytes (24-26) as well as in transgenic mice (27)) have identified a liver-specific enhancer located between positions –220 and –110 relative to the transcription start site. An additional region (between positions –595 to –192), which partially overlaps with the liver-specific enhancer, appears to control the intestine-specific expression of apoAI mRNA (28). Indeed, more recent work in carefully constructed wild-type and mutant lines of transgenic mice has suggested that the distal enhancer of the apoCIII gene may also play a significant modulatory role in some contexts (29, 30). While the effects of the distal apoCIII enhancer are most notable in the case of intestinal expression of the apoAI gene, it also influences apoAI gene transcription in hepatocytes to some extent (28, 30). Within the liver-specific enhancer, multiple transcription factor binding sites (designated site A (-214 to -192), site B (-169 to -146), and site C (-134 to 119)) have been identified (Figure 1B). Further, mutational analysis

revealed that maximal apoAI liver-specific enhancer activity depends upon the integrity of each of the sites, suggesting that synergistic interactions between these factors and other components of the various transcription complexes are responsible for apoAI gene expression (25, 31, 32).

Tissue specificity of many genes whose expression is restricted to the liver is imparted by combinatorial interactions between liver-enriched and ubiquitous transcription factors (33, 34). Thus, sites A and C serve as binding sites for many nuclear receptors (35-37) (see also below). For example, site A binds the retinoid receptors, RAR and RXRalpha the peroxisome proliferator activated receptor (PPARalpha) and the orphan receptors, ARP-1, COUP-TF1, and HNF-4. Of these, HNF-4, a liver- and intestine-enriched transcription factor appears to be primarily responsible for the maintenance-levels of apoAI gene expression in hepatocytes. This has become particularly evident from the detailed characterization of mice in which the gene encoding HNF-4 has been knocked out by homologous recombination (38, 39). Although this results in embryonic lethality (38), use of tetraploid embryos, which permits essentially normal fetal liver development, indicated that apoAI expression is abolished in *hnf4*$^{-/-}$ livers (39). Conversely, transgenic mice, which have been engineered to express human apoAI under the control of the various regulatory elements, were shown to express reduced levels of this protein when a mutated version of site A (which fails to bind HNF-4) was present in the corresponding construct (30). Furthermore, expression of apoAI in cultured hepatic and intestinal cells was strongly down-regulated upon infection with an adenovirus vector that delivered a dominant-negative derivative of HNF-4 (40).

Site B binds FoxA/HNF-3beta also a hepatocyte-enriched factor (31, 32) and, potentially also the ubiquitous factors C/EBP and NFY (26, 41). Indeed, a subregion of the liver-specific enhancer containing site A and site B retains substantial enhancer activity in the hepatoblastoma cell line HepG2 (32). Furthermore, in non-hepatic cells (such as CV-1) supplementation with ectopic FoxA/HNF-3 and HNF-4 results in efficient enhancer activity in a site B- and site A-dependent manner (32). Together, these results have identified FoxA/HNF-3 and HNF-4 as key players in the liver-specific ground state expression of the apoAI gene. Intriguingly, many liver-specific genes show this kind of a joint dependence on FoxA/HNF-3 and HNF-4 for their expression (33, 34).

As discussed in further detail below, additional layers of control appear to be superimposed on this basic mechanism since the apoAI gene expression levels can be modulated by other factors not normally required for routine expression. This includes control through retinoids (via RXR and site A, (37, 42)) as well as through Egr-1 (via additional target sites located upstream of the enhancer) (43, 44) and as indicated, C/EBP (potentially via site B) (26, 43). In this regard, the substantial contribution of the HNF-4 binding site located in the distal apoCIII enhancer must be noted (30). Similarly, the Sp1 sites also located there have been demonstrated to upregulate apoAI transcription (30). (The functional analysis of an additional Sp1 site that overlaps one of the Egr-1 sites (44) remains to be characterized.)

4.2. Transcriptional regulation by FoxA/HNF-3 and HNF-4

As is characteristic of members of the steroid/nuclear receptor superfamily, HNF-4 contains a conserved DNA-binding domain (DBD, containing typical zinc finger motifs) and an extended "ligand binding domain (LBD)(45). The putative LBD contains the conserved activation domain (AF-2) (46, 47). The corresponding domain in several receptors has been implicated in interactions with various coactivators (18) that include Mediator (see below). That Mediator is in fact critical for HNF-4 function (involving direct physical interactions predominantly through the TRAP220 subunit) has also been recently demonstrated (48). Therefore, in so far as HNF-4 is a principal regulator of apoAI gene transcription, these results also implicate Mediator in the expression of this gene. (Whether HNF-4 contains an AF-1 activation domain (typically found toward the N-terminal end of some receptors) is presently controversial (46, 49). The C-terminus of HNF-4 contains a proline-rich domain that is dispensable for transcriptional activation *per se* (46) but may play a modulatory role in some situations (50).)

Despite purported identification of a family of small molecules that interact with HNF-4 (51), it remained uncertain for some time whether HNF-4 is regulated by a ligand. But a recent structural study (52) has found that HNF-4 constitutively exists in the conformation that is characteristic of active nuclear receptors and is in tight association with lipids (a mixture of saturated and cis-monounstaurated C14-C18 fatty acids) in lieu of a conventional ligand. This is consistent with earlier observations that mammalian HNF-4 is a potent activator of transcription both in vitro (46, 48) and in most cell-types (including S. cerevisae) that have been tested. This lack of requirement for a conventional ligand also fits nicely with the evolutionary antiquity of HNF-4 (53), which in turn argues for its role as a primordial antecedent of nuclear receptors.

FoxA/HNF-3 (including the alpha, beta and gamma isoforms) is a liver-enriched transcription factor that is involved in developmental and homeostatic control of many liver-specific genes (54). Interestingly, the three-dimensional winged-helix structure of FoxA/HNF-3 resembles a globular domain of the linker histone H5 (55), leading to the suggestion, and preliminary indications, that FoxA/HNF-3 may possess nucleosome binding properties that favor the nucleation of an enhancer complex on nucleosomal templates (56, 57). However, the precise mechanism(s) by which HNF-4 activates transcription, either on its own or in synergy with FoxA/HNF-3 (whether on the apoAI gene or, for that matter, other liver-specific genes), remains unknown.

4.3. Potential mechanisms for synergistic function of FoxA/HNF-3 and HNF-4 at the apoAI liver-specific enhancer

Based on the preceding background, it appears that HNF-4, FoxA/HNF-3, chromatin modifying cofactors,

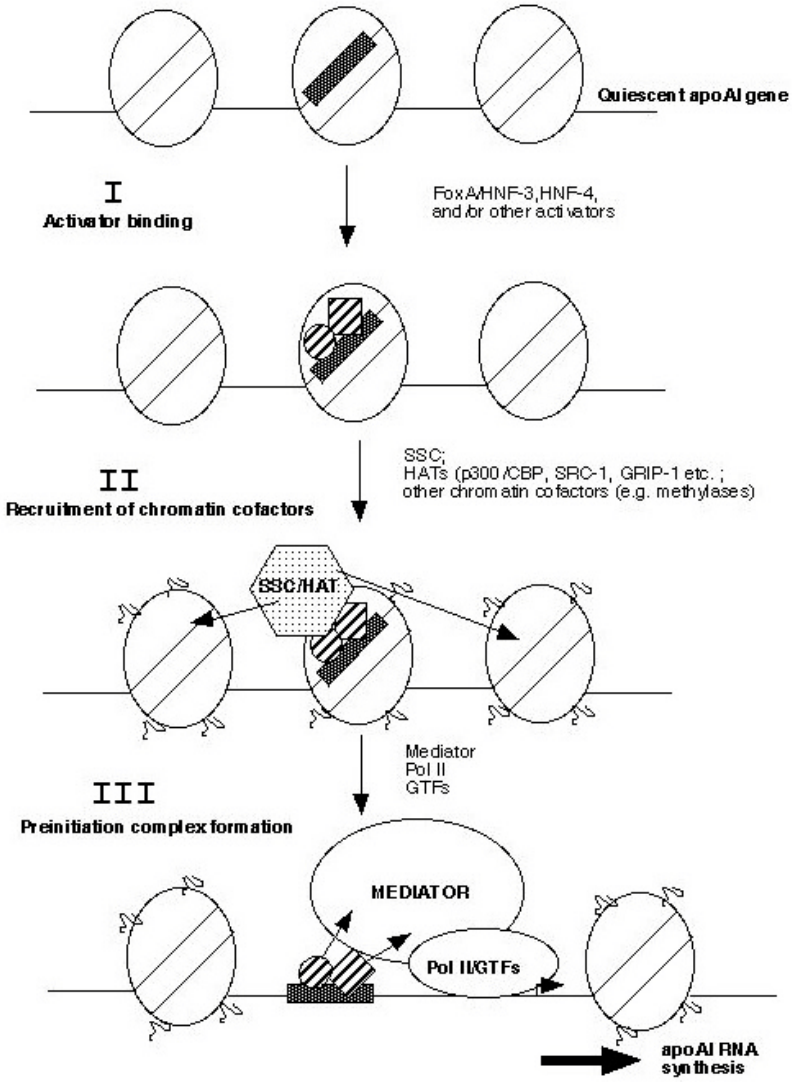

Figure 2. Multistep model for the activation of the apoAI gene. As explained in the text, figure indicates a hypothetical pathway for the onset of apoAI mRNA synthesis beginning with the quiescent apoAI gene situated in its presumed chromatin environment. Putative nucleosomes cover both the binding sites for various activators as well as the transcription start site. In step I, activators (FoxA/HNF-3 and HNF-4, shown as cross-hatched objects) bind to their cognate sites (speckled box) and initiate the activation process. They recruit various chromatin modifying complexes (SSC: Swi/SNF complex; HAT: histone acetyl transferase) which both remodel (reposition?) the nucleosomes as well as covalently modify (squiggly lines) the histones (step II). This allows the components of the preinitiation complex (Mediator, Pol II, GTFs) to be recruited, likely via direct interactions (arrows) of the enhancer-bound activators with distinct Mediator subunits (step III). Once in place, Pol II is able to commence RNA synthesis. Note that for simplicity only signals emanating from the liver-specific enhancer are shown. Nonetheless, activators bound to distal elements would also be expected to contribute at each of the steps, accounting for further synergistic effects.

Mediator and the Pol II-associated basal transcription machinery constitute the core of the mechanisms governing steady-state apoAI transcription in liver cells. Several models, incorporating current thinking on general gene activation mechanisms, potentially could provide coherence to these observations. First, both HNF-4 and FoxA/HNF-3, acting as conventional activators initially cooperate (synergistically) to recruit chromatin modifying coactivators. This would result in localized alteration of the chromatin structure, either via acetylation of the histone tails or through more generalized ATP-dependent remodeling of the nucleosomes. The resulting exposed apoAI core promoter region would then be accessible to Mediator, Pol II and the GTFs. Additional contributions towards synergy could come from separate interactions of HNF-4 and FoxA/HNF-3 with distinct Mediator subunits (and/or with distinct GTFs) at the level of the exposed DNA template (Figure 2).

Alternatively, given the involvement of FoxA/HNF-3 with chromatin (56, 57), this could be the factor primarily responsible for penetrating the chromatinized liver-specific enhancer (via its interaction with site B). It could thus nucleate the putative enhanceosome (below), and recruit critical chromatin-modifying factors. This is only to say that FoxA/HNF-3 effects may be dominant; given that HNF-4 also has been shown to physically and functionally interact with HATs such as p300/CBP (48, 58), and GRIP-1 (59) its contribution at the chromatin level cannot be overlooked. Once the chromatin structure has been altered through the action of FoxA/HNF-3, HNF-4 effects (via its interactions with Mediator and GTFs (including TFIIB) could predominate. This series of well-choreographed events could also manifest as the observed synergism between FoxA/HNF-3 and HNF-4. Nonetheless, overlapping models that account for the observations summarized above may also be constructed.

By analogy with other systems, it is likely that FoxA/HNF-3 and HNF-4 assemble into a well-defined, higher-order structure often termed the enhanceosome (60). Thus, it remains to be determined how this nucleoprotein assemblage is held together. However, typically, this involves (in addition to the primary transcription factors bound to their cognate sites on the template) architectural proteins such as those belonging to the high mobility group (HMG) (60). The net effect is the generation of a well-defined nucleoprotein complex, which potentially incorporates a number of signal-bearing transcription factors and likely functions in a stereospecific fashion to transduce their combined effects to the Pol II basal transcription complex.

5. REGULATION OF APOAI GENE TRANSCRIPTION IN RESPONSE TO VARIOUS STIMULI

While the above description entailing FoxA/HNF-3 and HNF-4 may likely be applicable to the maintenance-level expression of the apoAI gene in hepatocytes, it also is apparent that the gene responds to a wide range of developmental, hormonal and pharmacological signals (21). However, only a few of the original phenomenological findings have been studied at the molecular level. In all likelihood, these responses entail changes in the transcription factor makeup of the apoAI enhancer, which, except as noted below, remain largely uncharacterized.

5.1. Transcription factors involved in diverse responses

Historically, identification of ARP-1, a member of the COUP-TF subfamily of orphan nuclear receptors (35), as a protein that bound to site A of the apoAI liver-specific enhancer, provided the first clues that apoAI gene transcription may be under the control of nuclear receptors. As summarized above, it quickly became apparent that HNF-4 is primarily responsible for the steady state expression of apoAI. Nonetheless, the observation that site A can potentially interact with multiple members of the nuclear receptor superfamily opened the way for investigating the contribution of diverse factors. Note, parenthetically, that site A apparently is a composite binding site that consists of three half-sites of the consensus nuclear receptor binding site; within site A, these are arrayed in overlapping DR0 and DR1 configurations (25, 35).

Perhaps most notable among the factors implicated in apoAI gene transcription by this line of investigation was the receptor for retinoid X, or RXR (37). Given that ARP-1 and RXR bind to the common site A, but have opposite functional consequences – repression by ARP-1 and activation by RXR – it was proposed that prior repression by ARP-1 (and likely other COUP-TF family members) is required for subsequent RXR-mediated activation (42). Although this hypothesis is yet to be rigorously tested, a very practical consequence of this connection has been that it predicts a positive modulatory effect of the RXR ligand, retinoid X (and perhaps of other retinoids as well), on apoAI gene transcription. Indeed, exposure of cultured primary hepatocytes to retinoids resulted in elevated apoAI levels, although this has been attributed, in part, to as-yet uncharacterized effects at the post-transcriptional level (61). Nonetheless, targeted inactivation of the RXRalpha gene in hepatic cells was found to enhance apoAI gene transcription (62), possibly reflecting a primary role of RXRalpha as a dimerization partner of other nuclear receptors that appear to down-regulate apoAI gene expression in rodents (see also below).

PPARalpha involvement in apoAI originally became apparent from the observation that fibrates elevated apoAI expression levels, including in transgenic mice carrying the human apoAI gene and its regulatory sequences (63, 64, and references therein). The ultimate target of this large class of compounds is PPARalpha (which also binds site A of the human apoAI gene) although they do not represent the ligand *per se* for this nuclear receptor. The species-specific response to fibrates is noteworthy: in contrast to the situation in humans, rodent apoAI gene activity is down-regulated by fibrates (63). It appears that the three nucleotide difference in the site A sequences of the human and rodent (rat) genes renders the latter unable to bind PPARalpha in conjunction with RXRalpha, its heterodimerization partner. This may be because the rat regulatory region contains an additional site for the orphan receptor Rev-erb near the TATA box, which has negative effects on the transcription of the rat apoAI gene (63).

Although there is some controversy surrounding the issue, several studies (including epidemiological correlations) have implicated estrogen (estradiol) in regulating plasma HDL levels (65). For the most part, estrogen is thought to have direct positive effects on apoAI transcription levels in humans (66). Indeed, detailed analysis (67) revealed that in cultured cells of human hepatic origin, transcription of the apoAI gene was significantly enhanced. This response could be narrowed down to the –256 to +397 region of the apoAI gene (which includes the liver-specific enhancer) even though there is no discernable binding site (estrogen response element) for the estrogen receptor (ER).

On the other hand, animal studies (especially one in which cynomolgus monkeys were administered ethinyl estradiol (68)) have shown reduction in plasma apoAI and corresponding HDL levels. Furthermore, exposure to estrogen of a cell-line of hepatic origin, which has been engineered to overexpress ERalpha, resulted in diminution of apoAI gene transcription (69). Interestingly, this effect was offset by supplying an excess of ectopic RIP140 (70), which was originally isolated as a putative estrogen receptor coactivator. Since this effect appeared to be independent of detectable binding of ER to the apoAI liver-specific enhancer DNA, it was proposed that ER-mediated interference of apoAI transcription results from partitioning away of common cofactors by ER (69). In the end, however, it may well be that the effects of estrogen are indirect (e.g. through the induction of an additional gene product).

Perhaps the most dramatic transcriptional response of the apoAI gene that has thus far been documented involves the nephrotic syndrome (71). Injection of transgenic mice carrying the human apoAI gene under the control of the liver-specific enhancer with nephrotoxic serum led to increased apoAI gene transcription. Furthermore, consistent with a steep rise in Egr-1 concentration (but not of HNF-4) in hepatic extracts, an equivalent increase in apoAI gene expression was not seen in mice with an *Egr-1*$^{-/-}$ background.

Curiously, it had earlier been observed that Egr-1, which is only transiently expressed in certain pathophysiologic states of the liver (44), regulates apoAI transcription via specific cognate sites in the liver-specific enhancer, which are distinct from transcription factor binding sites discussed above (44). Unlike some other transcription factors, Egr-1 was able to up-regulate apoAI transcription regardless of the (presumptive) prevailing transcription factor configuration at the enhancer (43, 44). Thus, it was proposed that, by overriding preexisting controls, Egr-1 provides a mechanism for sustained apoAI expression during conditions of cellular stress such as those prevailing during liver regeneration. In retrospect, the newly uncovered role of Egr-1 in the nephrotic response of the apoAI gene (71) may be another manifestation of this phenomenon.

Reduction in apoAI gene expression may also account, at least in part, for hypocholesterolemia that accompanies an inflammatory reponse (72). Indeed, cytokines, such as TNFalpha, IL-1 and IL-6, which are believed to be mediators of inflammation, down-regulate the expression of the apoAI gene (among others) in cultured hepatic cells (72). Although at this point, the precise molecular mechanism by which this may be brought about remains unclear, it is likely that the relatively recently described family of signal transducing transcription factors called SMADs (73), which represent the end-point of cytokine signaling pathways, play a role in this process. Further insights may be derived by considering the effects of the anti-inflammatory cytokine, TGFbeta, on apoCIII gene expression (74). In this case, HNF-4 is subject to direct interaction with activated (phosphorylated) SMAD3 and SMAD4, which may lead, albeit indirectly, to enhanced transcriptional activation even though there are no cognate DNA binding sites for them in this regulatory region. While no similar data are presently available for the apoAI gene, by analogy with the apoCIII gene, with which the apoAI gene is co-regulated (including through HNF-4), a possible linkage with the SMAD pathway can reasonably be anticipated.

5.2. Potential mechanisms underlying the changing transcriptional responses of the apoAI gene

The above-cited examples of the transcriptional responses of the apoAI gene serve to illustrate the dynamic range of the regulatory apparatus governing apoAI gene expression. While the present examples of stimuli that impinge on the apoAI gene were selected because of the availability of accompanying data that shed some light on the underlying molecular mechanisms, it should be emphasized that a detailed mechanistic understanding is far from complete. Thus, for example, while both RXRalpha and Egr-1 may be involved in specific situations, it is still unclear, from a mechanistic standpoint, how these factors would be incorporated into the transcriptional machinery that drives apoAI gene expression. One would predict, *a priori*, that as these factors become mobilized and are recruited to the liver-specific enhancer, profound changes in the putative pre-existing enhanceosome ensue. Assuming that the default-state of the enhancer carries HNF-4 at site A, this implies that HNF-4 is first cast off and is then replaced by the incoming transcriptional regulator. However, a more significant aspect of this factor exchange, especially in conjunction with the above models for synergism among transcription factors (Figure 2), is the wholesale rearrangement of coactivator interactions that would also necessarily result. Outstanding issues concern whether the accompanying changes will include involvement of a fresh set of chromatin remodeling coactivators or re-establishment of contacts with (perhaps different subunits of) the Mediator.

What is clear is that these observations imply that the multi-component nucleoprotein complexes that constitute the putative enhanceosome and drive apoAI gene transcription are likely to be in a relative state of flux. The precise factor composition of the enhancer would thus be continually changing in response to diverse signals such as developmental messages (including hormones) and pharmacological and toxic substances. In turn, this built-in flexibility would afford the cell an opportunity to periodically fine-tune its production of apoAI.

6. CONCLUSIONS AND PERSPECTIVES

In this review, a wide range of transcription factors that have been implicated in the expression of the apoAI gene, either directly or indirectly, have been discussed. Essentially, two interrelated themes emerge from this brief overview. First, that powerful nucleoprotein assemblages (chromatin, enhanceosomes, multiprotein coactivator complexes) maintain constitutively high levels of apoAI gene expression in the appropriate cell-types. Second, that these assemblages are unlikely to be static in

that all evidence points to a remarkable capacity to assimilate incoming signal-bearing molecules. No doubt, future work, entailing new techniques developed in the transcription field, will be directed at obtaining a more detailed understanding of this system with its potential for a significant impact on therapies for cardiovascular disease in the longterm.

7. ACKNOWLEDGEMENTS

The author is indebted to Dr. S.K.Karathanasis for introducing him to the nuances of the apoAI system and for insights into its transcriptional regulation; and to Dr. R.G.Roeder for continued support and encouragement.

8. REFERENCES

1. Segrest, J. P., L. Li, G. M.Anantharamaiah, S. C. Harvey, K. N. Liadaki, & V. Zannis: Structure and function of apolipoprotein A-I and high-density lipoprotein. *Curr Opin Lipidol* 11, 105-115 (2000)
2. Segrest, J. P., S. C. Harvey, & V. Zannis: Detailed molecular model of apolipoprotein A-I on the surface of high- density lipoproteins and its functional implications *Trends Cardiovasc Med* 10, 246-252 (2000)
3. Karathanasis, S. K.: Lipoprotein metabolism: high-density lipoprotein *Monogr Hum Genet* 14, 140-171 (1992)
4. Rubin, E. M., R. M. Krauss, E. A. Spangler, J. G. Verstuyft, & S. M. Clift: Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI, *Nature* 353, 265-267 (1991)
5. Pajkrt, D., J. E. Doran, F. Koster, P. G. Lerch, B. Arnet, T. van der Poll, J. W. ten Cate, & S. J. van Deventer: Antiinflammatory effects of reconstituted high-density lipoprotein during human endotoxemia. *J Exp Med* 184, 1601-1608 (1996)
6. Naar, A. M., B. D. Lemon, & R. Tjian: Transcriptional coactivator complexes. *Ann Rev Biochem* 70, 475-501 (2001)
7. Malik, S. & R. G.Roeder: Transcriptional regulation through Mediator-like coactivators in yeast and metazoan cells. *Trends Biochem Sci* 25, 277-283 (2000)
8. Roeder, R. G.: Role of general and gene-specific cofactors in the regulation of eukaryotic transcription. *Cold Spring Harb Symp Quant Biol* 63, 201-218 (1998)
9. Tjian, R. & T. Maniatis: Transcriptional activation: a complex puzzle with few easy pieces. *Cell* 77, 5-8 (1994) .
10. Smale, S. T.: Core promoters: active contributors to combinatorial gene regulation. *Genes Dev* 15, 2503-2508 (2001)
11. Roeder, R. G.: The role of general initiation factors in transcription by RNA polymerase II. *Trends Biochem Sci* 21, 327-335(1996)
12. Orphanides, G., T. Lagrange, & D. Reinberg: The general transcription factors of RNA polymerase II. *Genes Dev* 10, 2657-2683 (1996)
13. Brivanlou, A. H. & J. E. Darnell, Jr.: Signal transduction and the control of gene expression. *Science* 295, 813-818 (2002)
14. Lee, T. I. & R. A. Young: Transcription of eukaryotic protein-coding genes. *Annu Rev Genet* 34, 77-137 (2000) .
15. Myers, L. C. & R. D. Kornberg: Mediator of transcriptional regulation. *Annu Rev Biochem* 69, 729-749 (2000)
16. Brown, C. E., T. Lechner, L. Howe, & J. L. Workman: The many HATs of transcription coactivators, *Trends Biochem Sci* 25, 15-19 (2000)
17. Stallcup, M. R., D. Chen, S. S. Koh, H. Ma, Y. H.Lee, H. Li, B. T. Schurter, & D. W. Aswad: Co-operation between protein-acetylating and protein-methylating co-activators in transcriptional activation. *Biochem Soc Trans* 28, 415-418 (2000)
18. Glass, C. K. & M. G. Rosenfeld: The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev* 14, 121-141 (2000)
19. Karathanasis, S. K., V. I. Zannis, & J. L. Breslow: Isolation and characterization of the human apolipoprotein A-I gene. *Proc Natl Acad Sci U S A* 80, 6147-6151 (1983)
20. Karathanasis, S. K., J. McPherson, V. I. Zannis, & J. L. Breslow: Linkage of human apolipoproteins A-I and C-III genes. *Nature* 304, 371-373 (1983)
21. Karathanasis, S. K.: Lipoprotein metabolism: high density lipoprotein. *Monogr Hum Genet* 14, 140-171 (1992)
22. Zannis, V. I., H. Y. Kan, A. Kritis, E. E. Zanni, & D. Kardassis: Transcriptional regulatory mechanisms of the human apolipoprotein genes in vitro and in vivo, *Curr Opin Lipidol* 12, 181-207 (2001)
23. Zannis, V. I., H. Y. Kan, A. Kritis, E. Zanni, & D. Kardassis, Transcriptional regulation of the human apolipoprotein genes. *Front Biosci* 6, D456-504 (2001)
24. Sastry, K. N., U. Seedorf, & S. K. Karathanasis: Different cis-acting DNA elements control expression of the human apolipoprotein AI gene in different cell types. *Mol Cell Biol* 8, 605-614 (1988) .
25. Widom, R. L., J. A.Ladias, S. Kouidou, & S. K. Karathanasis: Synergistic interactions between transcription factors control expression of the apolipoprotein AI gene in liver cells. *Mol Cell Biol* 11, 677-687 (1991)
26. Papazafiri, P., K. Ogami, D. P. Ramji, A. Nicosia, P. Monaci, C. Cladaras, & V. I. Zannis: Promoter elements and factors involved in hepatic transcription of the human ApoA-I gene positive and negative regulators bind to overlapping sites. *J Biol Chem* 266, 5790-5797 (1991)
27. Walsh, A., Y. Ito, & J. L. Breslow: High levels of human apolipoprotein A-I in transgenic mice result in increased plasma levels of small high density lipoprotein (HDL) particles comparable to human HDL3. *J Biol Chem* 264, 6488-6494 (1989)
28. Ginsburg, G. S., J. Ozer, & S. K.Karathanasis: Intestinal apolipoprotein AI gene transcription is regulated by multiple distinct DNA elements and is synergistically activated by the orphan nuclear receptor, hepatocyte nuclear factor 4. *J Clin Invest* 96, 528-538 (1995) .
29. Walsh, A., N. Azrolan, K. Wang, A. Marcigliano, A. O'Connell, & J. L. Breslow: Intestinal expression of the human apoA-I gene in transgenic mice is controlled by a DNA region 3' to the gene in the promoter of the adjacent convergently transcribed apoC-III gene. *J Lipid Res* 34, 617-623 (1993)
30. Kan, H. Y., S. Georgopoulos, & V. Zannis: A hormone response element in the human apolipoprotein CIII (ApoCIII) enhancer is essential for intestinal expression of

the ApoA-I and ApoCIII genes and contributes to the hepatic expression of the two linked genes in transgenic mice. *J Biol Chem* 275, 30423-30431 (2000) .

31. Harnish, D. C., S. Malik, & S. K. Karathanasis: Activation of apolipoprotein AI gene transcription by the liver- enriched factor HNF-3. *J Biol Chem* 269, 28220-28226 (1994)

32. Harnish, D. C., S. Malik, E. Kilbourne, R. Costa, & S. K. Karathanasis: Control of apolipoprotein AI gene expression through synergistic interactions between hepatocyte nuclear factors 3 and 4. *J Biol Chem* 271, 13621-13628 (1996) .

33. Lai, E. & J. E. Darnell, Jr. Transcriptional control in hepatocytes: a window on development. *Trends Biochem Sci* 16 427-430 (1991)

34. Xanthopoulos, K. G. & J. Mirkovitch: Gene regulation in rodent hepatocytes during development, differentiation and disease. *Eur J Biochem* 216, 353-60 (1993) .

35. Ladias, J. A. & S. K. Karathanasis: Regulation of the apolipoprotein AI gene by ARP-1, a novel member of the steroid receptor superfamily. *Science* 251, 561-565 (1991)

36. Tzameli, I. & V. I. Zannis: Binding specificity and modulation of the ApoA-I promoter activity by homo- and heterodimers of nuclear receptors. *J Biol Chem* 271, 8402-8415 (1996)

37. Rottman, J. N., R. L. Widom, B. Nadal-Ginard, V. Mahdavi, & S. K. Karathanasis: A retinoic acid-responsive element in the apolipoprotein AI gene distinguishes between two different retinoic acid response pathways. *Mol Cell Biol* 11, 3814-3820 (1991)

38. Chen, W. S., K. Manova, D. C. Weinstein, S. A. Duncan, A. S. Plump, V. R. Prezioso, R. F. Bachvarova, & J. E. Darnell, Jr.: Disruption of the HNF-4 gene, expressed in visceral endoderm, leads to cell death in embryonic ectoderm and impaired gastrulation of mouse embryos. *Genes Dev* 8, 2466-2477 (1994)

39. Li, J., G. Ning, & S. A. Duncan: Mammalian hepatocyte differentiation requires the transcription factor HNF-4alpha. *Genes Dev* 14, 464-474 (2000) .

40. Fraser, J. D., D. Keller, V. Martinez, D. Santiso-Mere, R. Straney, & M. R. Briggs: Utilization of recombinant adenovirus and dominant negative mutants to characterize hepatocyte nuclear factor 4-regulated apolipoprotein AI and CIII expression. *J Biol Chem* 272, 13892-13898 (1997)

41. Novak, E. M. & S. P. Bydlowski: NFY transcription factor binds to regulatory element AIC and transactivates the human apolipoprotein A-I promoter in HEPG2 cells. *Biochem Biophys Res Commun* 231, 140-143 (1997)

42. Widom, R. L., M. Rhee, & S. K. Karathanasis: Repression by ARP-1 sensitizes apolipoprotein AI gene responsiveness to RXR alpha and retinoic acid. *Mol Cell Biol* 12, 3380-3389 (1992)

43. Ge, R., M. Rhee, S. Malik, & S. K. Karathanasis: Transcriptional repression of apolipoprotein AI gene expression by orphan receptor ARP-1. *J Biol Chem* 269, 13185-13192 (1994) .

44. Kilbourne, E. J., R. Widom, D. C.Harnish, S. Malik, & S. K. Karathanasis: Involvement of early growth response factor Egr-1 in apolipoprotein AI gene transcription. *J Biol Chem* 270, 7004-7010 (1995)

45. Sladek, F. M.: Orphan receptor HNF-4 and liver-specific gene expression. *Receptor* 4, 64-68 (1994)

46. Malik, S. & S. K. Karathanasis TFIIB-directed transcriptional activation by the orphan nuclear receptor hepatocyte nuclear factor 4. *Mol Cell Biol*16, 1824-1831 (1996)

47. Hadzopoulou-Cladaras, M., E. Kistanova, C. Evagelopoulou, S. Zeng, C. Cladaras, & J. A. Ladias: Functional domains of the nuclear receptor hepatocyte nuclear factor 4. *J Biol Chem* 272, 539-550 (1997)

48. Malik, S., A. E. Wallberg, Y. K. Kang, & R. G. Roeder: TRAP/SMCC/mediator-dependent transcriptional activation from DNA and chromatin templates by orphan nuclear receptor hepatocyte nuclear factor 4. *Mol Cell Biol* 22, 5626-5637 (2002) .

49. Green, V. J., E. Kokkotou, & J. A. Ladias: Critical structural elements and multitarget protein interactions of the transcriptional activator AF-1 of hepatocyte nuclear factor 4. *J Biol Chem* 273, 29950-29957 (1998)

50. Sladek, F. M., M. D. Ruse, Jr., L. S. Nepomuceno, M. Huang, & M. R. Stallcup: Modulation of transcriptional activation and coactivator interaction by a splicing variation in the F domain of nuclear receptor hepatocyte nuclear factor 4alpha1. *Mol Cell Biol* 19, 6509-6522 (1999)

51. Hertz, R., J. Magenheim, I. Berman, & J. Bar-Tana: Fatty acyl-CoA thioesters are ligands of hepatic nuclear factor-4alpha. *Nature* 392, 512-516 (1998)

52. Wisely, G. B., A. B. Miller, R. G. Davis, A. D. Thornquest, R. Johnson, T. Spitzer, A. Sefler, B. Shearer, J. T. Moore, A. B. Miller, T. M. Wislon, & S. P. Williams: Hepatocyte nuclear factor-4 is a transcription factor that constitutively binds fatty acids, *Structure* 10, 1225-1234 (2002) .

53. Sluder, A. E. & C. V. Maina: Nuclear receptors in nematodes: themes and variations. *Trends Genet* 17, 206-213 (2001)

54. Hromas, R. & R. Costa: The hepatocyte nuclear factor-3/forkhead transcription regulatory family in development, inflammation, and neoplasia. *Crit Rev Oncol Hematol* 20, 129-140 (1995) .

55. Clark, K. L., E. D. Halay, E. Lai, & S. K. Burley: Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. *Nature* 364, 412-420 (1993)

56. Cirillo, L. A. & K. S. Zaret: An early developmental transcription factor complex that is more stable on nucleosome core particles than on free DNA. *Mol Cell* 4, 961-969 (1999)

57. Cirillo, L. A., F. R. Lin, I. Cuesta, D. Friedman, M. Jarnik, & K. S. Zaret: Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. *Mol Cell* 9, 279-289 (2002)

58. Dell, H. & M. Hadzopoulou-Cladaras: CREB-binding protein is a transcriptional coactivator for hepatocyte nuclear factor-4 and enhances apolipoprotein gene expression. *J Biol Chem* 274, 9013-9021 (1999)

59. Wang, J. C., J. M. Stafford, & D. K. Granner: SRC-1 and GRIP1 coactivate transcription with hepatocyte nuclear factor 4. *J Biol Chem* 273, 30847-30850 (1998)

60. Carey, M.: The enhanceosome and transcriptional synergy. *Cell* 92, 5-8 (1998)

61. Kaptein, A., D. M. Neele, J. Twisk, H. F. Hendriks, T. Kooistra, & H. M. Princen: Mechanism of the slow induction of apolipoprotein A-I synthesis by retinoids in cynomolgus hepatocytes: involvement of retinoic acid and retinoid X receptors. *J Lipid Res* 38, 2273-2280 (1997)

62. Wan, Y. J., D. An, Y. Cai, J. J. Repa, T. Hung-Po Chen, M. Flores, C. Postic, M. A. Magnuson, J. Chen, K. R. Chien, S. French, D. J. Mangelsdorf, & H. M. Sucov: Hepatocyte-specific mutation establishes retinoid X receptor alpha as a heterodimeric integrator of multiple physiological processes in the liver. *Mol Cell Biol* 20, 4436-4444 (2000)
63. Vu-Dac, N., S. Chopin-Delannoy, P. Gervois, E. Bonnelye, G. Martin, J. C. Fruchart, V. Laudet, & B. Staels: The nuclear receptors peroxisome proliferator-activated receptor alpha and Rev-erbalpha mediate the species-specific regulation of apolipoprotein A-I expression by fibrates. *J Biol Chem* 273, 25713-25720 (1998)
64. Hennuyer, N., P. Poulain, L. Madsen, R. K. Berge, L. M. Houdebine, D. Branellec, J. C. Fruchart, C. Fievet, N. Duverger, & B. Staels: Beneficial effects of fibrates on apolipoprotein A-I metabolism occur independently of any peroxisome proliferative response. *Circulation* 99, 2445-2451 (1999)
65. Rich-Edwards, J. W. & C. H. Hennekens: Postmenopausal hormones and coronary heart disease. *Curr Opin Cardiol* 11, 440-446 (1996)
66. Brinton, E. A.: Oral estrogen replacement therapy in postmenopausal women selectively raises levels and production rates of lipoprotein A-I and lowers hepatic lipase activity without lowering the fractional catabolic rate. *Arterioscler Thromb Vasc Biol* 16, 431-440 (1996)
67. Lamon-Fava, S., J. M. Ordovas, & E. J. Schaefer: Estrogen increases apolipoprotein (apo) A-I secretion in hep G2 cells by modulating transcription of the apo A-I gene promoter. *Arterioscler Thromb Vasc Biol* 19, 2960-2965 (1999)
68. Clarkson, T. B., C. A., Shively, T. M., Morgan, D. R., Koritnik, M. R. Adams, & J. R. Kaplan: Oral contraceptives and coronary artery atherosclerosis of cynomolgus monkeys. *Obstet Gynecol* 75, 217-222 (1990)
69. Harnish, D. C., M. J. Evans, M. S. Scicchitano, R. A. Bhat, & S. K. Karathanasis: Estrogen regulation of the apolipoprotein AI gene promoter through transcription cofactor sharing. *J Biol Chem* 273, 9270-9278 (1998)
70. Cavailles, V., S. Dauvois, F. L'Horset, G. Lopez, S. Hoare, P. J. Kushner, & M. G. Parker: Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *EMBO J* 14, 3741-3751 (1995)
71. Zaiou, M., N. Azrolan, T. Hayek, H. Wang, L. Wu, M. Haghpassand, B. Cizman, M. P. Madaio, J. Milbrandt, J. B. Marsh, J. L. Breslow, & E. A. Fisher: The full induction of human apoprotein A-I gene expression by the experimental nephrotic syndrome in transgenic mice depends on cis-acting elements in the proximal 256 base-pair promoter region and the trans-acting factor early growth response factor 1. *J Clin Invest* 101, 1699-1707 (1998)
72. Ettinger, W. H., V. K., Varma, M., Sorci-Thomas, J. S. Parks, R. C. Sigmon, T. K. Smith, & R. B. Verdery: Cytokines decrease apolipoprotein accumulation in medium from Hep G2 cells. *Arterioscler Thromb* 14, 8-13 (1994)
73. Attisano, L. & J. L. Wrana: Signal transduction by the TGF-beta superfamily. *Science* 296, 1646-1647 (2002)
74. Kardassis, D., K. Pardali, & V. I. Zannis: SMAD proteins transactivate the human ApoCIII promoter by interacting physically and functionally with hepatocyte nuclear factor 4. *J Biol Chem* 275, 41405-41414 (2000)

Key Words: Apolipoprotein AI; orphan nuclear receptors; HNF-4; HNF-3; coactivators; transcriptional synergism; enhancer; liver-specific transcription; review.

Send correspondence to: Dr Sohail Malik, Laboratory of Biochemistry and Molecular Biology, Rockefeller University, 1230 York Ave, New York, NY 10021, Tel: 212-327-7605, Fax: 212-327-7949, E-mail: maliks@rockvax.rockefeller.edu