

Pit-1 Determines Cell Types during Development of the Anterior Pituitary Gland

A MODEL FOR TRANSCRIPTIONAL REGULATION OF CELL PHENOTYPES IN MAMMALIAN ORGANOGENESIS*

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Understanding the molecular mechanisms by which complex organs, containing distinct cell phenotypes, can arise from a single or a limited number of progenitor cells represents a central problem in mammalian biology. In this brief review, we consider the POU domain factor Pit-1 (also called GHF-1) and the anterior pituitary gland as a model to demonstrate the molecular mechanisms by which a tissue-specific transcription factor can act to determine both cell phenotypes and regulate proliferation of differentiated cell types.

Development of the Anterior Pituitary Gland

The primordium of the anterior pituitary gland first appears as an invagination in the somatic ectoderm immediately anterior to the anterior neuropore on embryonic day 8.5 (e8.5) in the mouse (Fig. 1B). As the head fold progresses, these cells make direct contact with the neuroectodermal cells in the only area where mesenchymal tissue is missing between neuroectoderm and somatic ectoderm. Concomitant with this event, organ commitment appears to occur as a pituitary marker, the α subunit of glycoprotein hormones (α GSU), is expressed in a posterior to anterior gradient in cells making contact with neuroectoderm (1). Subsequently, five distinct cell types, each characterized by the expression of a unique hormone, appear in highly spatial and temporal specific fashion (Fig. 1B), suggesting that distinct pathways regulate terminal differentiation of each cell type. These cell types (and their characteristic hormone) are: gonadotropes (follicle-stimulating hormone and luteinizing hormone), corticotropes (adrenocorticotrophic hormone), thyrotropes (thyroid-stimulating hormone), somatotropes (growth hormone), and lactotropes (prolactin).

Cell lineage studies suggest that cells, which at least temporarily expressed growth hormone, serve as precursors for lactotropes (2, 3). Furthermore, whereas somatotropes retain proliferative activity in the adult, mature lactotropes are thought to be postmitotic (3). Following the appearance of all five cell types, there is an intermingling of the distinct cell types, obscuring their initially spatially distinct genesis (Fig. 1A).

Identification and Expression of Pit-1

Pit-1 is a transcription factor restricted to the anterior pituitary gland. Comparison of the predicted amino acid sequence of Pit-1 with that of the octamer binding proteins Oct-1 and Oct-2, as well as with the *Caenorhabditis elegans* developmental regulator unc-86, revealed that all contained a conserved homeodomain (the POU homeodomain) linked to another conserved domain referred to as the POU-specific domain (POUs).¹ The two domains are joined

together by a short poorly conserved linker sequence and together they are referred to as the POU (Pit-1, Oct-1, Oct-2, unc-86) domain (Fig. 2A). After the initial definition of this gene family, several additional POU domain genes, many with prominent expression throughout the nervous system, have been isolated (reviewed in Refs. 4 and 5).

With the isolation of the genes encoding the related pituitary hormones growth hormone and prolactin, the molecular basis of their regulation at a transcriptional level was investigated. These studies led to the identification of several AT-rich elements in the regulatory region of both growth hormone and prolactin genes. DNA-protein binding studies suggested that a protein unique to cell lines expressing the growth hormone and prolactin genes bound to these regions and that this factor(s) was able to initiate transcription from both genes *in vitro*. The biological relevance of these observations was tested in transgenic mouse models, which confirmed the importance of these regulatory regions for normal developmental activation of the growth hormone and prolactin genes (6, 7). Based on the DNA-binding site, Pit-1 was cloned (8, 9) and shown to control transcription from both the prolactin and growth hormone promoters (9-12).

Analyses of Pit-1 expression revealed that initiation of its expression correlated both spatially and temporally to activation of its distal target genes (1, 13). Thus, *Pit-1* is selectively activated in the caudomedial part of the nascent gland, preceding activation of prolactin, growth hormone, and thyroid-stimulating hormone β (TSH β) genes in this region (1). Immunohistological analysis revealed high expression in three of the five cell types, somatotropes, lactotropes, and thyrotropes. Further, defects in the *Pit-1* gene (the Snell and Jackson dwarfs) are characterized by absent growth hormone, prolactin, and TSH β gene expression and result in a failure of somatotrope, lactotrope, and thyrotrope proliferation (14). The initial appearance of thyrotropes in the developing gland on e12.5 in the rostral tip precedes, and is spatially distinct from, *Pit-1* gene activation in the caudomedial gland on e14.5. Analyses of the ontogeny of TSH β gene expression in wild type and *Pit-1* mutant mammals showed that the two populations of thyrotropes arise independently (15). The rostral tip population appears normally in the Snell dwarf, indicating that Pit-1 is not required for initial activation of the TSH β gene in these cells. However, these cells apparently disappear around birth (15). The caudomedial thyrotropes, which arise around e16.5, fail to appear in *Pit-1*-defective animals, indicating that Pit-1 is required for activation of TSH β gene expression in these cells. The localization of Pit-1 protein to these cells as well as the identification of Pit-1-binding sites in the TSH β promoter and demonstration that these sequences are Pit-1 responsive further suggest that the TSH β gene is a direct target for Pit-1 (1, 15-17).

Although most of our understanding of the role of *Pit-1* in pituitary development has come from rodent animal models, it is now known that Pit-1 carries out similar functions in humans. This is supported by the demonstration that humans with mutations in the *Pit-1* gene have a syndrome of congenital hypothyroidism, dwarfism, and prolactin deficiency (18-21), which is analogous to the phenotype of the Snell and Jackson dwarf mice.

Pit-1 as a Transcription Factor: Protein-DNA Interactions and Transactivation

The DNA-binding domain of Pit-1 is bipartite, composed of a 60-amino acid homeodomain that is linked with a non-conserved linker (15 amino acids) to the POU-specific domain, a 75-amino acid sequence (Fig. 2A). Structure of the POU homeodomain is similar to the helix-turn-helix structure of classic homeodomains. The three-dimensional NMR solution structure of the Oct-1 POU domain was recently reported to be homologous to the helix-turn-helix structure of λ repressors (22, 23). Based on these structural studies, as well as the recent crystal structure of the Oct-1 POU domain bound to

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The abbreviations used are: POU, POU-specific domain; TSH β , thyroid-stimulating hormone β ; GRF, growth hormone-releasing factor; GRFR, GRF receptor; kb, kilobases; GH, growth hormone.

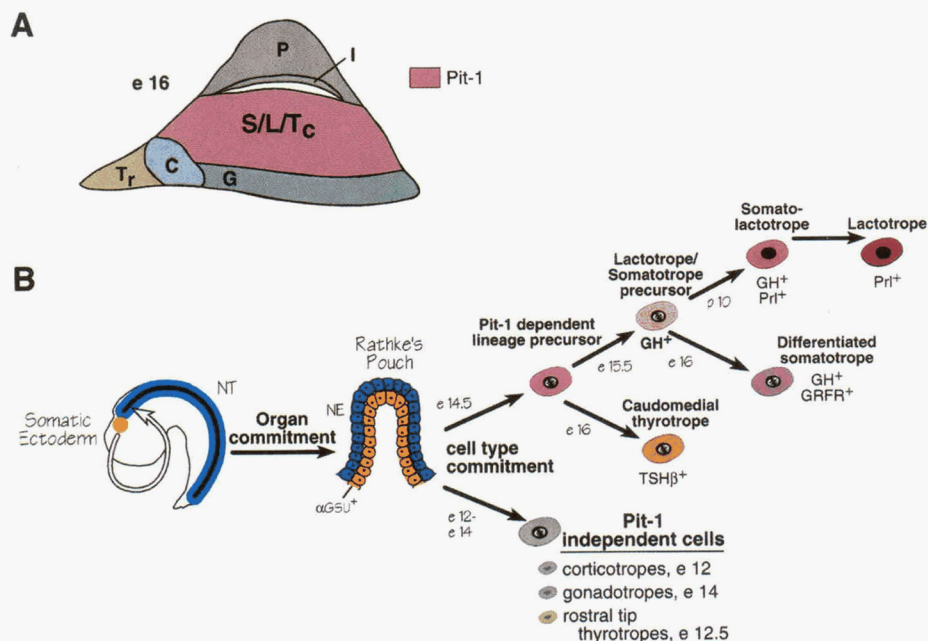


FIG. 1. Spatial and temporal regulation of Pit-1 expression. A, expression of Pit-1 is spatially regulated during embryogenesis. Pit-1 transcripts and Pit-1-dependent cell types appear in the central region of the developing anterior pituitary gland. Later these cells become distributed among other cell types throughout the adult pituitary gland. The Pit-1 expressing zone is denoted with a pink color. C, corticotropes; G, gonadotropes; I, intermediate lobe of the pituitary gland; L, lactotropes; P, posterior lobe of the pituitary gland; S, somatotropes; T_c, caudomedial thyrotropes; T_r, rostral tip thyrotropes. B, Pit-1 appears during the cell-type commitment phase of anterior pituitary development. Early in embryogenesis a region of somatic ectoderm (shown in yellow) comes in direct contact with neuroectoderm (NE, shown in blue), and an invagination referred to as Rathke's pouch forms. During this organ commitment phase, the somatic ectoderm cells are committed to the pituitary fate as indicated by expression of the α subunit of glycoprotein hormones (α GSU). Pit-1 transcripts appear in the central region of the developing anterior pituitary gland on embryonic day 14.5 (e14.5) in the mouse. Because the first known Pit-1 target, GH, appears 24 h later we have proposed the existence of a Pit-1-dependent lineage precursor cell. This precursor cell type appears to have two fates because it can give rise to either a GH-producing cell (lactotrope/somatotrope precursor) or caudomedial thyrotropes that express TSH. The lactotrope/somatotrope precursor can either differentiate to mature somatotropes, which are characterized by high expression of GH and the GRFR or to lactotropes that appear mainly after birth. The somato-lactotropes are proposed to be an intermediate cell type between the GH expressing somato-lactotrope precursor and the prolactin (Prl) expressing mature lactotropes. In addition to TSH β , the caudomedial thyrotropes express the α subunit common to glycoprotein hormones, and their relationship to the α subunit expressing gonadotropes is unknown. The indicated time points refer to mice where delivery occurs on embryonic day 19. NT, neural tube.

an octamer site (24), it is likely that each subdomain of the Pit-1 POU domain makes independent major groove contacts (Fig. 2C). This model is supported by studies on the binding of Pit-1 to response elements in the prolactin and growth hormone genes, demonstrating that both POU homeodomain and POU_s domain are required for high affinity binding (25). Thus, binding affinity of a protein lacking the POU_s domain for native Pit-1 response elements is decreased by more than 2 orders of magnitude. While high affinity DNA-binding sites for Pit-1 contain a core sequence, TATNCAT, or highly related octamer sites, these elements in the growth hormone and prolactin regulatory regions also contain an AT-rich stretch immediately 5' to the TATNCAT sequence (Fig. 2B). Pit-1 binds cooperatively to these response elements as a dimer, which requires the POU_s domain and which appears to be an important aspect of gene activation (25, 26).

Mutations through the TATNCAT sequence obliterate binding whereas mutations in the 5' AT-rich region prevent dimer formation on the site and decrease binding affinity 5–10-fold (27). Interestingly, when the TATNCAT sequence in Prl-1P was changed to an octamer site, Pit-1 did not form dimers on the site and affinity was decreased 8–10-fold. Furthermore, Pit-1 was ineffective in transactivating through this site, although other sites with similar binding affinity for Pit-1 allowed transactivation in the same system. This site, however, was transcriptionally active in cells containing Oct-2 (27). Thus lack of transactivation does not correlate entirely with changes in binding affinity, and subtle differences in the binding site must act to restrict the ability of Pit-1 to transactivate, in concert with a model suggesting that the DNA site itself acts as a modulator of Pit-1 action. The mechanisms by which the binding site alters the ability of Pit-1 to transactivate could reflect selective interactions of accessory proteins or alteration of Pit-1 structure by the binding site, which in turn modulates the ability of Pit-1 to interact with the basic transcriptional machinery. It is pertinent that potent transactivation by Pit-1 correlates with the ability of Pit-1 to dimerize on response elements.

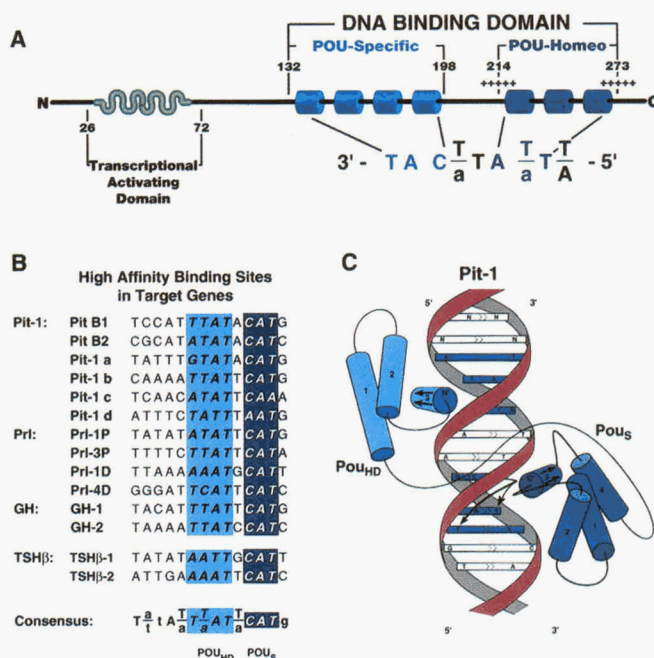


FIG. 2. Pit-1 and DNA-binding sites. A, a schematic of the Pit-1 protein. A transcriptional activation domain (shown in green) is localized to the NH₂ terminus. The POU domain localized between amino acids 132 and 273 is responsible for DNA binding. This domain is composed of the POU specific and POU homeodomain. The α helices are shown with the cylinders. Basic (+++++) regions in the POU domain are indicated. The consensus DNA-binding site with proposed protein contacts is shown below. B, alignment of high affinity DNA-binding sites from the GH, prolactin (Prl), Pit-1, and TSH β genes is shown. C, a model of the three-dimensional structure of the POU domain of Pit-1 and its interaction with a DNA-binding site.

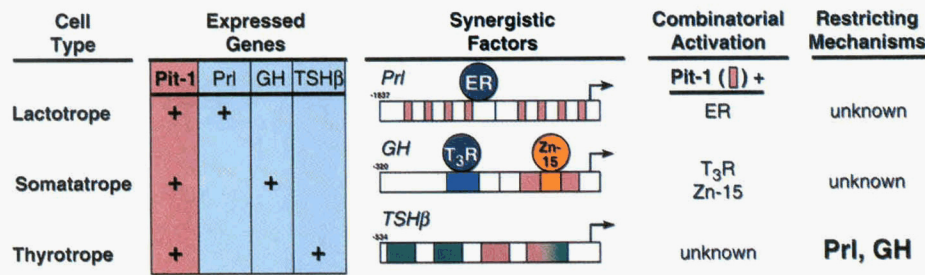


FIG. 3. **Restriction of Pit-1 action.** Although Pit-1 is expressed in lactotrope, somatotrope, and thyrotrope, these cell types selectively express the prolactin (*Prl*), GH, and TSH β genes, respectively. Because Pit-1 can bind to and activate each one of these genes, a mechanism must exist to allow Pit-1 to have distinct function in each cell type. This may be achieved through a combinatorial code where other factors that are relatively cell-specific can synergize with Pit-1. Examples of such factors are the estrogen receptor (*ER*) that binds to the prolactin regulatory region, the thyroid hormone receptor (*T₃R*), and Zen-15 (*Zn-15*) that bind to the GH regulatory region. In addition, "inappropriate" genes may be actively silenced in each cell type. There is evidence from experiments in transgenic mice for active restriction of prolactin reporter genes out of thyrotrope, mediated by defined cis-active elements (7).

Deletion analyses and transfer to heterologous DNA-binding domains show that a major transactivation domain of Pit-1 resides in amino acids 8–80 (25, 28, 29). However, there is evidence that the POU domain also participates in transactivation because of studies with a naturally occurring splice variant in which the POU domain is deleted, referred to as Δ 4Pit-1. A DNA site was identified that bound both wild type Pit-1 and Δ 4Pit-1 with equivalent affinity, but only Pit-1 holoprotein was able to serve as an activator on binding to this site (26). The mechanism whereby the POU domain affects transactivation remains unclear. It could alter the presentation of the NH₂-terminal transactivation domain or function as an independent transactivation domain that synergizes with the NH₂ terminus, or it might recruit other proteins required for transcription, similar to the recruitment of VP-16 by the Oct-1 POU homeodomain on selective DNA-responsive elements (30–32).

In the rat pituitary, the Pit-1 protein appears in two major forms, with relative molecular masses of 31 and 33 kDa, as a consequence of alternative usage of translation initiation sites (33). Several additional variants of Pit-1, resulting from alternative splicing, have been described (34–39). Because of the low abundance of all the variant Pit-1 proteins and the lack of demonstration of selectivity in their expression among normal somatotrope, lactotrope, and thyrotrope, the biological significance of these splice variants remains unclear.

Because Pit-1 is critical for transcription of pituitary genes encoding hormones that are under strict regulation by hypothalamic factors, there has been considerable interest to determine whether the Pit-1 protein is the target of this regulation. Several studies have showed that the Pit-1-binding sites in the prolactin gene regulatory region are required for control of prolactin gene transcription by thyrotropin-releasing hormone, calcium, dopamine, cAMP, and phorbol esters (40–45). Similarly, Pit-1-binding sites in the TSH β and growth hormone genes can mediate thyrotropin-releasing hormone and activin responsiveness, respectively (16, 46, 47). The Pit-1 protein is phosphorylated at two distinct sites in response to treatment with cAMP and phorbol esters. Phosphorylation of one of these sites, located in the homeodomain (Thr²²⁰), can alter the ability of Pit-1 to bind to certain DNA-binding sites (48, 49). Collectively, these studies suggest that in addition to a developmental role, the Pit-1 protein may play a role in a more transient regulation of gene expression.

Regulation of Proliferation and the Appearance of Anterior Pituitary Gland Cell Types by Pit-1

Identification of Pit-1 based on binding to the regulatory regions of the growth hormone and prolactin genes as well as the ability of Pit-1 to activate these genes and the TSH β gene suggested that all three genes are direct targets for Pit-1 (10, 12, 15–17). The lack of expression of these three genes in Pit-1-defective mice and humans (18–21) is consistent with this hypothesis. However, the failure of proliferation of thyrotrope, somatotrope, and lactotrope cell types in the Snell dwarf indicates that Pit-1 is also critical for proliferation and/or survival of these three cell types (14). A plausible model is that Pit-1 regulates the critical molecules that mediate proliferation. In this regard, based on studies of control of somatotrope growth, the growth hormone releasing factor (GRF) receptor was considered a potential Pit-1 target gene that was required for so-

matotrope cell proliferation. Cloning of the GRF receptor (GRFR) and demonstration of failure to express the GRFR transcript in the Snell mouse might now be consistent with such a model (50–52). As suggested by previous studies of GRF responsiveness, the *little* mouse was found to harbor a mutation in the GRF receptor, controlling GRF-mediated signal transduction (53, 54). Somatotrope in the pituitary of the *little* mouse are decreased by 90%, and this appears to be due to selective failure of proliferation in the centrally located (GRF-dependent) somatotrope population. In contrast to the Snell mouse, lactotrope and thyrotrope cell populations are normal in the *little* mouse. Because of the absence of all three cell types in the Snell mouse, it is presumed that Pit-1 also regulates genes encoding trophic factors and/or their receptors that are required for proliferation of lactotrope and thyrotrope.

Restriction of Pit-1 Action

Because Pit-1 protein is expressed in three of five pituitary cell types, yet each of the three distal target genes (prolactin, growth hormone, and TSH β) is restricted to single cell types, there must be additional mechanisms that limit the activity of Pit-1 in a cell-type specific fashion (Fig. 3). Two types of mechanisms, active suppression of target genes in heterologous cells and interaction with activating factors that enhance the effect of Pit-1 on selective genes, are suggested to underlie these cell-specific activities. Evidence for the former mechanism comes from studies in transgenic mice showing that sequences outside the Pit-1-binding sites in the prolactin gene were required for exclusion of these transcripts from thyrotrope (7). Whether similar suppressive mechanisms exist for the appropriate Pit-1 target genes in lactotrope and somatotrope remains to be determined.

Whereas the full nature of the mechanisms underlying synergistic gene activation in a cell-specific manner remains to be established (Fig. 3), there is evidence that the prolactin distal enhancer requires the estrogen receptor in addition to Pit-1 for full activity (11, 55, 56). Similarly, activity of the growth hormone promoter has been demonstrated to require several transcription factors in addition to Pit-1. These may include the thyroid hormone receptor and Zn 15, a novel zinc finger protein that binds between the Pit-1 elements in the growth hormone gene (57–62). It has been suggested that ligand-induced association of the thyroid hormone receptor with its cognate binding site may be a prerequisite for interaction of Pit-1 with the growth hormone gene regulatory region *in vivo* (63). Finally, synergy with an AP-1-like factor has been suggested in activation of the TSH β gene by Pit-1 (64).

Activation of the Pit-1 Gene

The mechanisms involved in activation of the *Pit-1* gene are of considerable interest because they may give insights into earlier steps in fate specification during organogenesis of the anterior pituitary gland. Studies using transgenic mice have shown that 14.8 kb of 5'-flanking sequence is sufficient to target expression to the anterior pituitary gland (65, 66). Further analyses, using defined pituitary cell lines, identified a 390-base pair enhancer located about 10 kb 5' to the transcription start site (66). This enhancer contains five Pit-1-binding sites, three of which were shown to be important for activation in transient transfection assays. The enhancer also contains a cell-specific element, a vitamin D₃ re-

sponse element, and a retinoic acid response element that mediates retinoic acid induction completely dependent on Pit-1. This provides an initial example of a POU domain factor-dependent retinoic acid response element. Because the *Pit-1* gene retinoic acid response element is distinct from known retinoic acid response elements, which contain a direct repeat with a spacing of 2 or 5 base pairs, this type of DNA site may permit unique regulation by retinoic acid of gene expression involved in differentiation (66).

Studies where 15 kb of *Pit-1* 5'-flanking sequence were linked to the SV40 large T oncogene led to tumor formation in mice (65). These tumors expressed Pit-1 but not growth hormone or prolactin, consistent with the possibility that these cells represent a somato-lactotrope progenitor cell. *In vitro* studies using cell lines derived from this tumor identified an enhancer between -3.1 and -5.3 that was less active in GC cells.

The prevalence of Pit-1-binding sites in the *Pit-1* enhancer suggests that autoregulation might be an important part of activation of the *Pit-1* gene. However, the initial activation of the *Pit-1* gene must be due to different mechanisms, as evident in the Snell dwarf, where the *Pit-1* gene is activated in a normal manner and is later (e18.5-p0) extinguished (66). The control region of the *Pit-1* gene, which is responsible for its initial activation, remains to be defined.

Conclusion

The anterior pituitary gland provides an example of mammalian organogenesis in which there appears first to be a commitment to organ identity early in development. Later, specific distinct cell phenotypes are established, each cell type arising in a distinct temporal and spatial fashion. A tissue-specific POU domain transcription factor, Pit-1, serves as a developmental regulator specifying three of the five anterior pituitary cell types, requiring both restriction and synergy with other transcription factors to achieve these cell-specific effects. In addition to activation of genes encoding distal phenotypic markers (hormones), Pit-1 apparently can also regulate genes required for proliferation of these cell types. Similar strategies may be used by other transcription factors that regulate cell differentiation and organ development.

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REFERENCES

- Simmons, D. M., Voss, J. W., Ingraham, H. A., Holloway, J. M., Broide, R. S., Rosenfeld, M. G., and Swanson, L. W. (1990) *Genes & Dev.* **4**, 695-711
- Behringer, R. R., Mathews, L. S., Palmiter, R. D., and Brinster, R. L. (1988) *Genes & Dev.* **2**, 453-461
- Borrelli, E., Heyman, R. A., Arias, C., Sawchenko, P. E., and Evans, R. M. (1989) *Nature* **339**, 538-541
- Andersen, B., Schonemann, M. D., Pearce, R. V., II, Jenne, K., Sugarman, J., and Rosenfeld, M. G. (1993) *J. Biol. Chem.* **268**, 23390-23398
- Rosenfeld, M. G. (1991) *Genes & Dev.* **5**, 897-907
- Lira, S. A., Crenshaw, E. B., III, Glass, C. K., Swanson, L. W., and Rosenfeld, M. G. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 4755-4759
- Crenshaw, E. B., III, Kalla, K., Simmons, D. M., Swanson, L. W., and Rosenfeld, M. G. (1989) *Genes & Dev.* **3**, 959-972
- Bodner, M., Castrillo, J. L., Theill, L. E., Deerinck, T., Ellisman, M., and Karin, M. (1988) *Cell* **55**, 505-518
- Ingraham, H. A., Chen, R., Mangalam, H. J., Elsholtz, H. P., Flynn, S. E., Lin, C. R., Simmons, D. M., Swanson, L., and Rosenfeld, M. G. (1988) *Cell* **55**, 519-529
- Mangalam, H. J., Albert, V. R., Ingraham, H. A., Kapiloff, M., Wilson, L., Nelson, C., Elsholtz, H., and Rosenfeld, M. G. (1989) *Genes & Dev.* **3**, 946-958
- Day, R. N., Koike, S., Sakai, M., Muramatsu, M., and Maurer, R. A. (1990) *Mol. Endocrinol.* **4**, 1964-1971
- Fox, S. R., Jong, M. T. C., Casanova, J., Ye, Z.-S., Stanley, F., and Samuels, H. H. (1990) *Mol. Endocrinol.* **4**, 1069-1080
- Dollé, P., Castrillo, J.-L., Theill, L. E., Deerinck, T., Ellisman, M., and Karin, M. (1990) *Cell* **60**, 809-820
- Li, S., Crenshaw, E. B., III, Rawson, E. J., Simmons, D. M., Swanson, L. W., and Rosenfeld, M. G. (1990) *Nature* **347**, 528-533
- Lin, S.-C., Li, S., Drolet, D. W., and Rosenfeld, M. G. (1994) *Development* **120**, 515-522
- Steinfelder, H. J., Hauser, P., Nakayama, Y., Radovick, S., Taylor, T., Weintraub, B. D., and Wondisford, F. E. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 3130-3134
- Gordon, D. F., Haugen, B. R., Sarapura, V. D., Nelson, A. R., Wood, W. M., and Ridgway, E. C. (1993) *Mol. Cell. Endocrinol.* **96**, 75-84
- Ohta, K., Nobukuni, Y., Mitsubuchi, H., Fujimoto, S., Matsuo, N., Inagaki, H., Endo, F., and Matsuda, I. (1992) *Biochem. Biophys. Res. Commun.* **189**, 851-855
- Pfaffle, R. W., DiMattia, G. E., Parks, J. S., Brown, M. R., Wit, J. M., Jansen, M., van der Nat, H., van den Brande, J. L., Rosenfeld, M. G., and Ingraham, H. A. (1992) *Science* **257**, 1118-1121
- Radovick, S., Nations, M., Du, Y., Berg, L. A., Weintraub, B. D., and Wondisford, F. E. (1992) *Science* **257**, 1115-1118
- Tatsumi, K., Miyai, K., Notomi, T., Kaibe, K., Amino, N., Mizuno, Y., and Kohno, H. (1992) *Nature Genet.* **1**, 56-58
- Assa-Munt, N., Mortishire-Smith, R. J., Aurora, R., Herr, W., and Wright, P. E. (1993) *Cell* **73**, 193-205
- Dekker, N., Cox, M., Boelens, R., Verrijzer, C. P., van der Vliet, P. C., and Kaptein, R. (1993) *Nature* **362**, 852-855
- Klemm, J. D., Rould, M. A., Aurora, R., Herr, W., and Pabo, C. O. (1994) *Cell* **77**, 21-32
- Ingraham, H. A., Flynn, S. E., Voss, J. W., Albert, V. R., Kapiloff, M. S., Wilson, L., and Rosenfeld, M. G. (1990) *Cell* **61**, 1021-1033
- Voss, J. W., Wilson, L., Rhodes, S. J., and Rosenfeld, M. G. (1993) *Mol. Endocrinol.* **7**, 1551-1560
- Elsholtz, H. P., Albert, V. R., Treacy, M. N., and Rosenfeld, M. G. (1990) *Genes & Dev.* **4**, 43-51
- Theill, L. E., Castrillo, J.-L., Wu, D., and Karin, M. (1989) *Nature* **342**, 645-648
- Ding, Y., Lu, W., Roberson, M. S., Moye-Rowley, W. S., and Maurer, R. A. (1991) *Mol. Endocrinol.* **5**, 1239-1245
- Gerster, T., and Roeder, R. G. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 6347-6351
- Kristie, T. M., LeBowitz, J. H., and Sharp, P. A. (1989) *EMBO J.* **8**, 4229-4238
- Stern, S., Tanaka, M., and Herr, W. (1989) *Nature* **341**, 624-630
- Voss, J. W., Yao, T.-P., and Rosenfeld, M. G. (1991) *J. Biol. Chem.* **266**, 12832-12835
- Konzak, K. E., and Moore, D. D. (1992) *Mol. Endocrinol.* **6**, 241-247
- Morris, A. E., Kloss, B., McChesney, R. E., Bancroft, C., and Chasin, L. A. (1992) *Nucleic Acids Res.* **20**, 1355-1361
- Theill, L. E., Hattori, K., Lazzaro, D., Castrillo, J.-L., and Karin, M. (1992) *EMBO J.* **11**, 2261-2269
- Day, R. N., and Day, K. H. (1994) *Mol. Endocrinol.* **8**, 12-20
- Day, R. N., and Day, K. H. (1994) *Mol. Endocrinol.* **8**, 374-381
- Haugen, B. R., Wood, W. M., Gordon, D. F., and Ridgway, E. C. (1993) *J. Biol. Chem.* **268**, 20818-20824
- Elsholtz, H. P., Lew, A. M., Albert, P. R., and Sundmark, V. C. (1991) *J. Biol. Chem.* **266**, 22919-22925
- Hoggard, N., Davis, J. R. E., Berwaer, M., Monget, P., Peers, B., Belayew, A., and Martial, J. A. (1991) *Mol. Endocrinol.* **5**, 1748-1754
- Iverson, R. A., Day, K. H., d'Emden, M., Day, R., and Maurer, R. A. (1990) *Mol. Endocrinol.* **4**, 1564-1571
- Yan, G.-z., Pan, W. T., and Bancroft, C. (1991) *Mol. Endocrinol.* **5**, 535-541
- Yan, G.-z., and Bancroft, C. (1991) *Mol. Endocrinol.* **5**, 1488-1497
- Peers, B., Monget, P., Nalda, M. A., Voz, M. L., Berwaer, M., Belayew, A., and Martial, J. A. (1991) *J. Biol. Chem.* **266**, 18127-18134
- Mason, M. D., Friend, K. E., Copper, J., and Shupnik, M. A. (1993) *Biochemistry* **32**, 8932-8938
- Struthers, R. S., Gaddy-Kurten, D., and Vale, W. W. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 11451-11455
- Kapiloff, M. S., Farkash, Y., Wegner, M., and Rosenfeld, M. G. (1991) *Science* **253**, 786-789
- Steinfelder, H. J., Radovick, S., and Wondisford, F. E. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 5942-5945
- Lin, C. R., Lin, S.-C., Chang, C.-P., and Rosenfeld, M. G. (1992) *Nature* **360**, 765-768
- Mayo, K. E. (1992) *Mol. Endocrinol.* **6**, 1734-1744
- Gaylinn, B. D., Harrison, J. K., Zysk, J. R., Lyons, C. E., Lynch, K. R., and Thorner, M. O. (1993) *Mol. Endocrinol.* **7**, 77-84
- Godfrey, P., Rahal, J. O., Beamer, W. G., Copeland, N. G., Jenkins, N. A., and Mayo, K. E. (1993) *Nature Genet.* **4**, 227-232
- Lin, S.-C., Lin, C. R., Gukovsky, I., Lusis, A. J., Sawchenko, P. E., and Rosenfeld, M. G. (1993) *Nature* **364**, 208-213
- Waterman, M. L., Adler, S., Nelson, C., Greene, G. L., Evans, R. M., and Rosenfeld, M. G. (1988) *Mol. Endocrinol.* **2**, 14-21
- d'Emden, M. C., Okimura, Y., and Maurer, R. A. (1992) *Mol. Endocrinol.* **6**, 581-588
- Ye, Z. S., Forman, B. M., Aranda, A., Pascual, A., Park, H. Y., Casanova, J., and Samuels, H. H. (1988) *J. Biol. Chem.* **263**, 7821-7829
- Schafele, F., West, B. L., and Reudelhuber, T. L. (1990) *J. Biol. Chem.* **265**, 17189-17196
- Schafele, F., West, B. L., and Baxter, J. D. (1992) *Mol. Endocrinol.* **6**, 656-665
- Lipkin, S. M., Naar, A. M., Kalla, K. A., Sack, R. A., and Rosenfeld, M. G. (1993) *Genes & Dev.* **7**, 1674-1687
- Lira, S. A., Kalla, K. A., Glass, C. K., Drolet, D. W., and Rosenfeld, M. G. (1993) *Mol. Endocrinol.* **7**, 694-701
- Tansey, W. P., Schafele, F., Heslewood, M., Handford, C., Reudelhuber, T. L., and Catanzaro, D. F. (1993) *J. Biol. Chem.* **268**, 14906-14911
- Force, W. R., and Spindler, S. R. (1994) *J. Biol. Chem.* **269**, 9682-9686
- Kim, M. K., McClaskey, J. H., Bodenner, D. L., and Weintraub, B. D. (1993) *J. Biol. Chem.* **268**, 23366-23375
- Lew, D., Brady, H., Klausung, K., Yaginuma, K., Theill, L. E., Stauber, C., Karin, M., and Mellon, P. L. (1993) *Genes & Dev.* **7**, 683-693
- Rhodes, S. J., Chen, R., DiMattia, G. E., Scully, K. M., Kalla, K. A., Lin, S.-C., Yu, V. C., and Rosenfeld, M. G. (1993) *Genes & Dev.* **7**, 913-932