Mini-Review

fos Protooncogene and the Regulation of Gene Expression in Adipocyte Differentiation

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CELLULAR development occurs in several stages in higher eukaryotic organisms. This process is generally characterized by the loss of pluripotency and restriction of possible cell fates, leading to a highly "determined" cell that can either proliferate or differentiate into a defined end-type cell. The process of cell differentiation can be studied in many cell types, some of which offer convenient culture systems. We are particularly interested in the development of fat cells. In our studies we have had two objectives. First, to use adipocyte differentiation as a model for the process of signal transduction in cellular development, whereby extracellular stimulation leads to the ultimate expression of end-product genes, extinction of genes characteristic of the precursor cells, and phenotypic differentiation. In addition, because the adipose cell is centrally involved in certain pathological syndromes of systemic energy balance, most notably obesity, obesity-linked diabetes, and cachexia (fat and muscle wasting), we are interested in using studies of gene control to improve our understanding of these disorders.

Because cancer is a disease involving loss of cellular growth control and disruption of the usual pattern of cell differentiation, there has been much interest in the role of oncogenes and their normal cellular counterparts (protooncogenes) in controlling patterns of gene expression during differentiation. Many studies using the expression of oncogenes introduced into cells have shown profound effects, either promoting or inhibiting differentiation depending on the oncogene and cellular system being analyzed (4, 5, 8, 13, 17, 19, 21, 24). One difficulty with this approach has been that the connection between the oncoprotein and the ultimate alterations in gene expression is usually not clear. We have recently approached the same goals through a different method. By studying in detail the *cis*- and *trans*-acting components involved in activating a particular gene during adipocyte differentiation, we have identified the first gene product related to an oncogene (*fos*) which appears to play a direct role in controlling gene expression during differentiation. This will be the major subject discussed below.

Adipose Differentiation in Cultured Cell Lines

The study of adipocyte development became much simpler with the isolation of preadipocyte cell lines that differentiate in culture, initially by Green and Kehinde (16) and later by many others. These lines typically resemble fibroblasts when kept in a growing state but undergo morphological and biochemical changes characteristic of fat cells when they stop growing under appropriate culture conditions. Many or most of the key enzymes of fatty acid and triglyceride synthesis are greatly increased during this process. The striking change in cell shape during adipocyte differentiation is independent of lipid accumulation; blocking triglyceride formation by depriving cells of biotin has little or no effect on the alterations in cell morphology (20). The gross changes in cell morphology are accompanied by alterations in the synthesis and assembly of cytoskeletal proteins (28). Experiments which interfered with morphological conversion using exogenous extracellular matrix components suggest that changes in cellular structure may be necessary for the programmed alterations in gene expression (29). The differentiation of adipocytes is accelerated by a number of defined or undefined factors including FCS, insulin, growth hormone, glucocorticoids, sex steroids, thyroid hormones, and indomethacin. Differentiation is inhibited by interferon, retinoic acid, interleukin-1, tumor promoters, and tumor necrosis factor. It is likely that at least some of these factors play a physiological role in adipocyte differentiation in vivo but this remains to be proven.

Novel Gene Products of Fat Cells

By any morphological or biochemical criteria, the change from a fibroblastic preadipocyte to an adipocyte is very dramatic. Early electrophoretic studies of preadipocyte and adipocyte proteins indicated that there were major quantitative changes in at least 100 protein species (27) and 40% of the soluble adipocyte proteins represented newly produced material (30). These include many of the important enzymes involved in fatty acid and triglyceride synthesis, such as (to name only a few) glycerophosphate dehydrogenase, fatty acid synthetase, phosphoenolpyruvate carboxykinase, acetyl CoA carboxylase, hormone-sensitive lipase, lipoprotein lipase, and malic enzyme. The electrophoretic studies cited above suggested that there are proteins produced during adipocyte differentiation in addition to the well known lipid-producing enzymes. This was clearly shown by the cloning and sequencing of several abundant, differentiation-dependent cDNAs. These include a novel homologue of myelin P2 protein, which also shares some NH_2-terminal sequence homology with a family of intracellular lipid carrier proteins that includes liver fatty acid–binding protein, intestinal heart

fatty acid-binding protein, and cellular retinoic acid-binding protein (1, 7). The precise function of this putative lipid carrier protein, termed adipocyte P2 (aP2)[1] or 422 protein remains to be determined. Another novel adipocyte gene product is the serine protease homolog adipsin, formerly called 28K (6). This protein, which has a catalytic "charge-relay" system and an activation peptide typical of serine proteases, is greatly altered quantitatively in certain models of obesity (6, 14). A third newly identified gene product of fat cells encodes a member of the cytochrome P-450 family (Ringold, G., personal communication). There is no reason to believe there are not many other important, adipocyte-specific gene products which await description and analysis. In light of the suggested roles of adipose tissue in the regulation of systemic energy balance, further studies may prove invaluable.

Regulation of Adipocyte Gene Expression

In virtually every case so far examined, new specific protein synthesis in the adipocyte is accompanied by increases in the related mRNA levels. Transcriptional analysis using isolated preadipocyte and adipocyte nuclei have shown that changes in transcription play a major role in altered mRNA abundance (2, 7, 12). Additionally, discordances between relative transcription rates and specific mRNA abundances have suggested that at least two fat cell mRNAs, aP2 and adipsin, might become especially abundant due to their great stability in the fat cell (7, 12).

The aP2 gene was chosen for further analysis of mechanisms controlling transcription because this gene is small (4 kb) and has a relatively simple structure (18). The entire aP2 gene, containing 2 kb of 5′ flanking sequence was stably introduced into 3T3-F442A preadipocytes after inserting a linker into a unique restriction site in the third exon (18). This marked gene was expressed only upon adipocyte differentiation and did so with a time course indistinguishable from the endogenous aP2 gene. We then began to examine particular sequences within this gene for regulatory properties. The isolation of several genes which are activated in adipocyte differentiation but belong to distinct protein families allowed a search for sequence homologies which might represent core elements involved in differentiation-dependent transcription. Two elements were described, which we termed "fat-specific elements" (FSEs, reference 18). FSE1 is a 14-base sequence which is present in multiple nonidentical copies in the 5′ flanking region of 3 genes: glycerophosphate dehydrogenase, aP2, and adipsin. FSE2 is present in a single copy on the 5′ flanking regions of only the glycerophosphate dehydrogenase and aP2 genes, matching at 13 out of 14 bases (18, 23). Since the sequence match was very close for the FSE2 element, we asked if this represented a protein binding site for a nuclear factor. Gel retardation analysis indicated that a sequence-specific protein or proteins interacted at this site (11). Furthermore, there was a very dramatic shift in mobility during differentiation; preadipose cells yielded a very broad streaking pattern while fat cells gave rise to a single distinct band.

There is also a change in apparent function of the FSE2 associated with the preadipose to adipose conversion. Only 168 bases of 5′ flanking sequence of the aP2 gene are required to drive differentiation-dependent expression of a heterologous marker gene, bacterial chloramphenicol acetyltransferase (CAT) in transient assays (11). When deletions from −144 to −120 removed the FSE2 site (located at −124), the expression of CAT was greatly increased in preadipocytes, whereas adipocyte expression decreased in this deletion. The FSE2 was also analyzed by cotransfecting a 50-fold molar excess of FSE2 duplex DNA (as a 28-mer) with the parent paP2-CAT (−168) plasmid. This also had the effect of increasing expression in preadipose cells but had no effect on fat cell expression. These data strongly suggest that the FSE2 functions as a negative regulator in preadipocytes and is recognized by a *trans*-acting factor. There is also a positive acting element near the FSE2 in fat cells, but whether it lies slightly upstream of FSE2 or includes the FSE2 is still unclear.

fos Involvement in aP2 Expression

The identification of a sequence associated with the preadipose to adipose conversion led to a study of the *trans*-acting complexes bound to the FSE2 site. Surprisingly, the FSE2 element turned out to be a target sequence for the binding of a protein complex involving the product of the *c-fos* protooncogene or other *fos*-like proteins (Fos). While binding of adipocyte-specific factors resided in a single band in a gel retardation assay, binding of preadipocyte factors appeared as a broad streak. The heterogeneity of the preadipocyte pattern suggested that the FSE2-binding complexes contained highly modified proteins. This hypothesis led us to test the notion that the nuclear protooncogene *c-fos*, known to be extensively and heterogeneously phosphorylated, might be part of this complex. While a precise sequence target of *c-fos* (or any other nuclear protooncogene) was unknown at this time, protein complexes containing this protein were known to bind to DNA and were suspected to function as *trans*-acting factors for genes involved in cell growth and differentiation (9). Using affinity-purified antibodies prepared against a *c-fos* peptide to disrupt nucleoprotein complexes, it became clear that Fos was present in the FSE2-binding material from both adipocytes and preadipocytes (11). This disruption of factor binding could be completely neutralized by prior adsorption of antibodies with the cognate *c-fos* peptide. Antibodies to other nuclear proteins had no effect on these complexes. The presence of Fos in the proteins bound to FSE2 could also be demonstrated by photo-cross-linking FSE2 complexes and immunoprecipitating with antibodies directed toward different parts of the *c-fos* molecule (10, 11, 26). A role for Fos in the sequence-specific binding was suggested by the fact that antibodies to Fos could disrupt preformed nucleoprotein complexes.

More recently, it has been shown that nuclear extracts enriched in either *v-* or *c-fos* show enhanced binding to the FSE2 DNA (26). Using a microscale DNA-affinity precipitation technique followed by two-dimensional electrophoresis, Franza et al. (15) have demonstrated directly the presence of *c-fos* protein and a number of Fos-related antigens in protein complexes that can bind to FSE2 and closely related DNA sequences.

Suggestions as to the function of Fos in the regulation of the aP2 gene have emerged through more detailed definition of its binding sequence. Mutation and sequence competition studies in vitro have shown that Fos complexes bind DNA

1. *Abbreviations used in this paper*: aP2, adipocyte P2; FSEs, fat-specific elements.

containing the sequence TGACTCA (15, 26), previously identified as the consensus sequence for the binding of mammalian transcription factor AP-1 (22) and yeast transcription factor GCN4 (31). AP-1 is a transcription factor which acts positively or negatively at a number of enhancers and is believed to play a role in enhancers stimulated by the phorbol ester tumor promoter, 12-0-tetradecanoyl phorbol-13-acetate (22). The simultaneous presence of proteins related to Fos and AP-1 in the same fat cell extracts was demonstrated by photo-cross-linking of FSE2 complexes and immunoprecipitation with antibodies to *c-fos* or to a protein closely related to AP-1, *v-jun* (26). AP-1 is closely related or identical to the cellular form of this oncogene, *c-jun*.

From these results emerged the first suggestion of a functional relationship between Fos and transcription factor AP-1. It is possible that Fos and AP-1 compete for binding and function as transcription factors/transcription inhibitors at related sequences. Alternatively, these results are also compatible with these proteins modulating each other's action directly, through a protein-protein interaction. Very recent evidence suggests that Fos and AP-1 may indeed form a stable complex which can bind to DNA in a sequence-specific manner (25). These data open several very intriguing questions. The first is whether Fos itself is a DNA-binding protein or is carried into this target sequence by virtue of its putative interaction with AP-1. The evidence that AP-1 is a DNA-binding protein is quite good; highly purified (though not homogeneous) preparations of AP-1 bind DNA in sequence-specific fashion (22), as do preparations of *v-jun* (3), a close relative of AP-1. The putative DNA binding domain of *v-jun* will substitute in a functional assay in yeast for a similar domain of the yeast transcription factor GCN4 (31). Although Fos protein complexes will bind DNA, there have been no reports of purified Fos binding to DNA in a sequence-specific manner, suggesting that Fos may well be bound to DNA through other proteins such as AP-1. On the other hand, Fos bound to FSE2 can be photo-cross-linked to the DNA indicating a close physical proximity (11). Antibodies to Fos disrupt native (noncross-linked) nucleoprotein complexes and cause the release of free DNA. Our perspective on this data is that AP-1 almost certainly is a DNA-binding protein and that Fos is very close to the DNA and is likely to influence the sequence-specific DNA-binding properties of complexes in which it participates. Also unknown is the significance of the multiple nuclear proteins which appear to be immunologically related to Fos and which also bind to the same or similar target sequences (15). Presumably they also play a role in the regulation of genes through binding at these target sequences.

Finally, a major question is how the differentiation-dependent changes in both the structure and function of FSE2 complexes on the aP2 gene are accomplished. Theoretically, this could involve the covalent modification of Fos or other proteins in this complex, or may represent the addition or subtraction of distinct proteins during the adipocyte differentiation process. Sequence-affinity purification of the FSE2 binding factors from preadipocyte and adipocytes, followed by assay using in vitro transcription systems will be required to answer this question. Reagents should soon be available to analyze Fos function in transcription in great detail. Given this precedent in adipocyte development, there is little doubt that *c-fos*, *c-jun*, and other nuclear protooncogenes participate in the regulation of other genes that are important in cell growth and development. Identifying and analyzing such genes will be an important future step in understanding normal and pathological development.

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References

1. Bernlohr, D. A., C. W. Angus, M. D. Lane, M. A. Bolanowski, and T. J. Kelly, Jr. 1984. Expression of specific mRNA during adipose differentiation: identification of an mRNA encoding a homologue of myelin P2 protein. *Proc. Natl. Acad. Sci. USA.* 81:5468-5472.
2. Bernlohr, D. A., M. A. Bolanowski, T. J. Kelly, Jr., and M. A. Lane. 1985. Evidence for an increase in transcription of specific mRNAs during differentiation of 3T3-L1 adipocytes. *J. Biol. Chem.* 260:5563-5567.
3. Bos, T. J., D. Bohmann, H. Tsuchie, R. Tjian, and P. K. Vogt. 1988. v-jun encodes a nuclear protein with enhancer binding properties of AP-1. *Cell.* 52:705-712.
4. Cherington, V., C. Gee, M. Brown, E. Paucha, B. Spiegelman, and T. Roberts. 1987. Analysis of the effects of polyoma and SV40 large T antigens on differentiation. *In* Nuclear Oncogenes. F. W. Alt, E. Harlow, and E. B. Ziff, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 138-143.
5. Cherington, V., B. Morgan, B. M. Spiegelman, and T. Roberts. 1986. Recombinant retroviruses that transduce individual polyoma tumor antigens: effects on growth and differentiation. *Proc. Natl. Acad. Sci. USA.* 83:4307-4311.
6. Cook, K. S., D. L. Groves, H. Y. Min, and B. M. Spiegelman. 1985. A developmentally regulated mRNA from 3T3 adipocytes encodes a novel serine protease homologue. *Proc. Natl. Acad. Sci. USA.* 82:6480-6484.
7. Cook, K. S., C. R. Hunt, and B. M. Spiegelman. 1985. Developmentally regulated mRNA in 3T3-adipocytes: analysis of transcriptional control. *J. Cell Biol.* 100:514-520.
8. Coppola, J. A., and M. D. Cole. 1986. Constitutive c-myc expression blocks mouse erythroleukemia cell differentiation but not commitment. *Nature (Lond.).* 320:760-763.
9. Curran, T. 1988. Fos. *In* The Oncogene Handbook. E. P. Reddy, A. M. Skalka, T. Curran, editors. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands. In press.
10. Distel, R., and B. M. Spiegelman. 1988. Involvement of fos as a trans-acting factor in adipogenic gene expression. *Prog. Clin. Biol. Res.* In press.
11. Distel, R. J., H.-S. Ro, B. S. Rosen, D. L. Groves, and B. M. Spiegelman. 1987. Nucleotide complexes that regulate gene expression in adipocyte differentiation: direct participation of c-fos. *Cell.* 49:835-844.
12. Doglio, A., C. Davi, P. Grimaldi, and G. Ailhaud. 1986. Growth hormone regulation of the expression of differentiation-dependent genes in preadipocyte ob1771 cells. *Biochem. J.* 238:123-129.
13. Falcone, G., F. Tato, and S. Alema. 1985. Distinctive effects of the viral oncogenes myc, erb, fps, and src on the differentiation pattern of quail myogenic cells. *Proc. Natl. Acad. Sci. USA.* 82:426-430.
14. Flier, J. S., K. S. Cook, P. Usher, and B. M. Spiegelman. 1987. Severely impaired adipsin expression in genetic and acquired obesity. *Science (Wash. DC).* 237:405-408.
15. Franza, B. R., Jr., F. J. Rauscher III, S. F. Josephs, and T. Curran. 1988. The fos complex and fos-related antigens recognize sequence elements that contain AP-1 binding sites. *Science (Wash. DC).* 239:1150-1153.
16. Green, H., and O. Kehinde. 1974. Sublines of mouse 3T3 cells that accumulate lipid. *Cell.* 1:113-116.
17. Grimaldi, P., D. Czerucka, M. Rassoulzadegan, F. Cuzin, and G. Ailhaud. 1984. ob17 cells transformed by the middle-T-only gene of polyoma virus differentiate in vitro and in vivo into adipose cells. *Proc. Natl. Acad. Sci. USA.* 81:5440-5444.
18. Hunt, C. R., J. H.-S. Ro, D. E. Dobson, H. Y. Min, and B. M. Spiegelman. 1986. Adipocyte P2 gene: developmental expression and homology of 5'-flanking sequences among fat cell-specific genes. *Proc. Natl. Acad. Sci. USA.* 83:3786-3790.
19. Kahn, P., L. Frykberg, C. Brady, I. Standly, H. Beug, B. Bennstrom, and T. Graf. 1986. V-erbA cooperates with sarcoma oncogenes in leukemic cell transformation. *Cell.* 45:349-356.
20. Kuri-Harcuch, W., L. S. Wise, and H. Green. 1978. Interruption of the adipose conversion of 3T3 cells by biotin deficiency: differentiation with-

out triglyceride accumulation. *Cell.* 14:53–59.
21. Lachman, H. M., and A. I. Skoultchi. 1984. Expression of c-myc changes during differentiation of mouse erythroleukaemia cells. *Nature (Lond.).* 310:592–594.
22. Lee, W., P. Mitchell, and R. Tjian. 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. *Cell.* 49:741–752.
23. Phillips, M., P. Djian, and H. Green. 1986. The nucleotide sequence of three genes participating in the adipose differentiation of 3T3 cells. *J. Biol. Chem.* 261:10821–10827.
24. Prochownik, E. V., and J. Kukowska. 1986. Deregulated expression of c-myc by murine erythroleukemia cells prevents differentiation. *Nature (Lond.).* 322:848–850.
25. Rauscher, F. J., III, D. R. Cohen, T. Curran, T. J. Bos, P. K. Vogt, D. Bohmann, R. Tjian, and B. R. Franza, Jr. 1988. Fos-associated p39 is the product of the jun proto-oncogene. *Science (Wash. DC).* 240: 1010–1016.
26. Rauscher, F. J., III, L. Sambuceti, T. Curran, R. J. Distel, and B. M. Spiegelman. 1988. Common DNA binding site for fos protein complexes and transcription factor AP-1. *Cell.* 52:471–480.
27. Sidhu, R. 1979. Two-dimensional electrophoretic analyses of proteins synthesized during differentiation of 3T3-L1 preadipocytes. *J. Biol. Chem.* 254:11111–11118.
28. Spiegelman, B., and S. Farmer. 1982. Decreases in tubulin and actin gene expression prior to morphological differentiation of 3T3 adipocytes. *Cell.* 29:53–60.
29. Spiegelman, B., and C. Ginty. 1983. Fibronectin modulation of cell shape and lipogenic gene expression in 3T3-adipocytes. *Cell.* 35:657–666.
30. Spiegelman, B., and H. Green. 1980. Control of specific protein biosynthesis during the adipose conversion of 3T3 cells. *J. Biol. Chem.* 255: 8811–8818.
31. Struhl, K. 1987. The DNA-binding domains of the jun oncoprotein and the yeast GCN4 transcriptional activator protein are functionally homologous. *Cell.* 50:841–846.