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Hypothesis

Regulatory squelching

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Abstract

An important function of transcription factors may be to sequester coactivators or corepressors of transcription. In this manner transcription factors could regulate in trans the activity of promoters to which they do not bind. This may be of widespread significance as a mechanism to control cell cycle-dependent and differentiation-specific transcriptional activity within eukaryotic cells. Therefore squelching in vivo may be more important than hitherto appreciated.

Key words: Transcription regulation; Transcription factor

Eukaryotic gene regulation can be viewed as an interplay between opposing activating and repressing influences. Transactivation of genes is controlled at many levels, but typically involves binding by transcription factors to specific regulatory sites within the promoter. Transactivation domains (TADs) within many transcription factors mediate interactions with other components affecting transcriptional initiation [1,2]. These TADs enable a factor not only to transactivate but also to squelch at higher concentrations. Squelching is repression of transcription by sequestering limiting components (e.g. coactivators) required for transcriptional activation away from the promoter in the affected gene [1,3]. Similar processes could also enhance transcription by sequestering repressors of transcription (Fig. 1).

Mechanistically, squelching of RNA polymerase II-driven transcription could operate at different levels. TADs could interfere with general components of the Pol II holoenzyme [4] such as TFIIB [5], or TFIID [6]. Interactions with TFIID could be at the level of the TATA-binding protein (TBP), or TBP associated factors (TAFs) [7–9]. Since different types of TFIID may exist, with both common and unique TAFs [9,10], squelching via a given TAF could potentially affect all TFIID-mediated transcription, or only a subset of promoters requiring that TAF. Thus squelching offers the potential for transcriptional repression at either a panoramic level, or restricted to subsets of genes whose promoters rely on the availability of specific factors not universally required. For instance the p53 protein can repress TATA box-mediated, but not initiator element-mediated transcription, by interacting with basal transcription factors [11], whereas acidic TADs squelch activated but not basal transcription [3]. Alternatively, the targets of squelching could be non-TAF coactivators which could affect interactions between histones or transcription factors and the promoter, or stabilize an active initiation complex by bridging between the nascent TATA-complex and DNA-bound transactivators [3,12–16]. The oncoprotein Bcl-3 provides an example of a non-TAF coactivator of NF-κB p50B homodimer-driven transcription [17].

We would like to draw attention to a potential level of regulation which has received scant consideration: namely that squelching may be a common mechanism employed by transcription factors to down-regulate promoters whose activity is governed by coactivators of low abundance. It is interesting to note that viruses often attack the cell at the level of coactivator imitation or exploitation, altering the transcriptional environment to one more conducive to their own growth [18–20]. That the cell is susceptible to this type of exploitation suggests that coactivator availability should indeed be crucial in determining the transcriptional profile of the cell. In the following discussion we document just a few cases which suggest that squelching may be a regulatory mechanism within eukaryotic cells.

The estrogen hormone receptor was shown to inhibit the transcriptional activation mediated by the progesterone and glucocorticoid receptors. This inhibition was independent of the DNA-binding and dimerization domain of the estrogen receptor and could be attributed to

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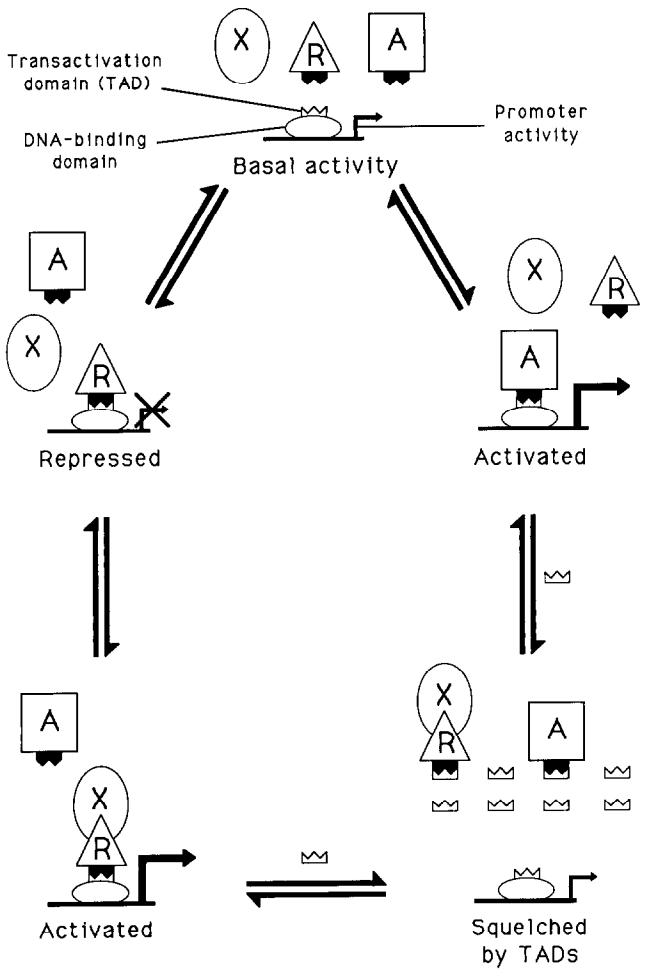

Fig. 1. Corepressors may compete with coactivators to influence transcription. Schematized representation of a DNA-bound transcription factor interacting with a corepressor (R) or coactivator (A). A corepressor could block transactivation, or recruit one or more additional coactivating factors (X) to transactivate. Such a hypothetical group of coactivators acting in series has been referred to as a 'coactivator cascade' [12]. An excess of TADs can squelch activated transcription. Although depicted as interacting directly with the TAD, a corepressor could conceivably bind elsewhere to sterically prevent coactivator recruitment, or to allosterically affect the affinity of the TAD for the coactivator. While represented as affecting only activated transcription, squelching could also affect basal levels by sequestration of components of RNA Pol II holoenzyme (e.g. [11]).

both the N-terminal A/B and the C-terminal hormone binding domains, which both contain a TAD. Reciprocally, transactivation mediated by the estrogen receptor was inhibited by the progesterone or glucocorticoid receptors [21]. This inhibition, independent of the DNA-binding function, strongly suggests a competition for coactivators. These studies were dependent on the overexpression of steroid hormone receptors and might therefore be artifactual. However, it was shown in two breast cancer cell lines expressing solely endogenous steroid hormone receptors that estrogen-dependent transcription could also be blocked by the addition of agonistic ligands of the progesterone and glucocorticoid receptors. Furthermore, the antiprogesterone/antiglucocorticoid ligand RU486 alleviated this repression [21]. This indicates that, upon activation by interaction with agonistic ligands, a TAD localized in the hormone binding-domain may sequester coactivators required by other steroid receptors, and presumably additional factors.

The transcription factor AP-1 consists of members of the proto-oncoprotein families Jun and Fos [22]. Mutual antagonism between AP-1 and various steroid receptor transcription factors has been often observed [22–24] and in some instances is probably due to squelching. This was shown for c-Jun and c-Fos, which trans-repress estrogen receptor-activated transcription in transient transfection experiments. This is independent of the b-ZIP DNA-binding domain of c-Jun, but probably requires the TADs and therefore may involve squelching [25]. A coactivator activity from F9 embryonal carcinoma (EC) stem cells, necessary for transactivation by v-Jun, c-Jun and JunB, has been identified as a target of squelching by v-Jun and c-Jun [26].

Additionally to interference with steroid receptors, the AP-1 members c-Jun, JunB and c-Fos can effectively repress transactivation by the muscle specific proteins myogenin and MyoD in transfection experiments. However this is not so for JunD, which is constitutively expressed in muscle cells [27]. The majority of mutual repression between MyoD and c-Jun seems to occur via a direct interaction involving the leucine zipper of c-Jun and the helix–loop–helix motif of MyoD [28]. However deleting the c-Jun leucine zipper did not completely alleviate repression, and c-Fos also mediated repression yet did not interact with MyoD, implying that squelching could be involved. Similarly, McBride et al. [29] observed that exogenous c-Fos, c-Jun, Fra-1, FosB and v-Fos could all trans-repress the region of the *ANF* promoter which confers cardiac muscle specificity. This repression did not require an AP-1 binding site in the promoter, a b-ZIP domain in c-Jun or the C-terminus of c-Fos involved in repression of the c-*fos* promoter (see below). However the repressing molecules all possessed a functional TAD, suggesting that the TADs of c-Fos and c-Jun titrated away a non-abundant muscle-specific coactivator. Thus transcription factors with Fos- or Jun-like TADs can potentially trans-repress tissue specific expression independently of their DNA-binding or dimerization functions in transfection experiments, implying that squelching may regulate differentiation-specific transcription.

In quail fibroblasts, the transcription factor Myb can activate the HSP70 promoter independently of a Myb DNA-binding site in the promoter, or of the Myb DNA-binding domain upon transient transfection [30]. This could be due to the sequestering of a repressor. Myb can also activate a promoter containing a Myb binding site in the same cells. Activation requires the DNA-binding domain of Myb and, indicative of squelching, can be

suppressed by Myb overexpression independently of a DNA-binding domain in the suppressing molecule [31]. Further evidence suggesting the existence of squelching as a regulatory mechanism is provided by the existence of the B-Myc molecule. It is homologous to the N-terminal TAD of c-Myc but lacks a basic region and helix–loop–helix motif, and suppresses transactivation by c-Myc [32]. Such a protein, homologous only to a TAD, may function to inhibit transcriptional activation by other proteins with similar TADs. B-Myc may thus represent a 'regulatory squelching' factor.

Another case may involve the transcription factor NF-κB. The p50 and p65 subunits heterodimerise to activate promoters containing NF-κB binding sites. A naturally occurring splice variant of p65 has been identified (p65Δ) which is unable to dimerize or bind DNA, but contains a functional TAD, and whose mRNA is abundant in non-differentiated hematopoietic cells [33]. Transfection of p65Δ transformed fibroblasts and interfered with NF-κB-induced transcription upon T-cell activation, but only when the TAD was functional, leading to speculation that p65Δ somehow permits unrestricted proliferation [33]. While this work included non-transfected primary cells the mechanism of action of p65Δ remains unclear. The TAD is clearly important, so a squelching-type mechanism seems plausible.

The serum response element (SRE) is a promoter element which confers not only rapid induction but also repression to a variety of immediate early genes. It is bound by several proteins including the serum response factor (SRF) [34]. Introduction of an SRE in front of a reporter gene activates the reporter in non-induced NIH3T3 cells. This indicates that SRE-bound SRF mediates activation. However, even very low doses of cotransfected exogenous SRF expression plasmid lead to an SRE-dependent repression of transcription [35–37]. Therefore endogenous SRF may already be in the squelching region of its dose–response curve. Thus any up-regulation of the endogenous *SRF* gene might concomitantly lead to repression of promoters relying on SRF for activity, via squelching. Such an up-regulation occurs for instance after the induction of quiescent cells [38] and may contribute to the postinductional down-regulation of immediate early genes. Therefore this mode of squelching could be an important regulator of the transient transcription of immediate early genes in the transition from the G_0 to G_1 phases of the cell cycle.

Interestingly, it has been found that SRF activates transcription in reconstituted in vitro transcription reactions, but additionally exerts squelching at higher concentrations, possibly via a coactivator which is utilized not only by SRF but also by other factors such as VP16 [39]. If the Fos protein could also interact with this coactivator, it could explain how c-Fos represses the c-*fos* and several other immediate early gene promoters via SREs [34,40], namely by sequestering a coactivator required for SRF-dependent transcription. This type of repression is apparently independent of the DNA-binding function of c-Fos but requires the C-terminus of the protein [40–43] and therefore may involve squelching.

The retinoblastoma protein (Rb) and the related molecule p107 are examples of corepressors. They interact with E2F and can be recruited to promoters in a complex with E2F, thereby preventing E2F-mediated transactivation [44]. Not only do viral transforming proteins such as the adenovirus E1A protein imitate coactivator activities by binding to TFIID [20], they also activate E2F sites by binding to Rb and p107 [44], thereby depriving the cell of corepressors. Thus they attack the cell at precisely the levels at which we propose squelching may be able to regulate transcription.

Perspective

The observation that squelching can suppress muscle differentiation-specific transcription in cultured cells (see above) implies that this type of regulation is possible endogenously and may be widely relevant. For example, a potential strategy to differentiate cell lineages during development would be to express combinations of different but related molecules, whose absolute concentrations, and ratios relative to each other, could influence the availability of different transcriptional coactivators in separately differentiating cells. The *Hox* genes involved in embryo pattern formation [45] are ideal candidates for this type of regulation. We emphasize in this model that regulation of promoters bound by Hox proteins may not be the sole means by which members of the *Hox* gene family specify the differentiation status of a cell or tissue. The non-homologous regions of the proteins may selectively sequester a variety of coactivators, thus pleiotropically affecting the transcriptional activity of a broad range of genes in trans.

In conclusion, squelching could be a commonly occurring transcriptional regulatory mechanism. The potential for regulatory squelching by transcription factors is accompanied by a number of corollaries. (i) Sequestering of coactivators could in some cases be the most important function of a particular transcription factor. (ii) Any promoter whose activity is dependent on the availability of a coactivator could be influenced by the expression of a broad range of unrelated transcription factors. (iii) A transcription factor with several TADs could trans-repress some promoters via squelching with one TAD, while activating other promoters in cis using a different TAD. (iv) DNA-binding-independent transcriptional controls should be considered indispensable when assessing the phenotype imposed by a transcription factor. (v) Finally, this concept has serious ramifications for interpreting tissue culture and transgenic animal studies involving constitutive overexpression of transcription factors.

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References

[1] Ptashne, M. (1988) Nature 335, 683–689.
[2] Mitchell, P. and Tjian, R. (1989) Science 245, 371–378.
[3] Ptashne, M. and Gann, A.A.F. (1990) Nature 346, 329–331.
[4] Sawadago, M. and Sentenac, A. (1990) Annu. Rev. Biochem. 59, 711–754.
[5] Lin, Y.-C, Ha, I., Maldonado, E., Reinberg, D. and Green, M.R. (1991) Nature 353, 569–571.
[6] Pugh, B.F. and Tjian, R. (1992) J. Biol. Chem. 267, 679–682.
[7] Hisatake, K.S., Hasegawa, S., Takada, R., Nakatani, Y., Horikoshi, M. and Roeder, R.G. (1993) Nature 362, 179–181.
[8] Weinzierl, R.O.J., Dynlacht, B.D. and Tjian, R. (1993) Nature 362, 511–517.
[9] Gill, G., Pascal, E., Tseng, Z.H. and Tjian, R. (1994) Proc. Natl. Acad. Sci. USA. 91, 192–196.
[10] Brou, C.S., Chaudhary, S., Davidson, I., Lutz, Y., Wu, J., Egly, J.-M., Tora, L. and Chambon, P. (1993) EMBO J. 12, 489–499.
[11] Mack, D.H., Vartikar, J., Pipas, J.M. and Laimins, L.A. (1993) Nature 363, 281–283.
[12] Tasset, D., Tora, L., Fromental, C., Scheer, E. and Chambon, P. (1990) Cell 62, 1177–1187.
[13] Dynlacht, B.D., Hoey, T. and Tjian, R. (1991) Cell 66, 563–576.
[14] Tanese, N., Pugh, B.F. and Tjian, R. (1991) Genes Dev. 5, 2212–2224.
[15] Croston, G.E., Laybourn, P.J., Paranjape, S.M. and Kadonga, J.T. (1992) Genes Dev. 6, 2270–2281.
[16] Fujita, T., Nolan, G.P., Liou, H.-C., Scott, M.L. and Baltimore, D. (1993) Genes Dev. 7, 1354–1363.
[17] Bours, V., Franzoso, G., Azarenko, V., Park, S., Kanno, T., Brown, K. and Siebenlist, U. (1993) Cell 72, 729–739.
[18] Martin, K.J., Lillie, J.W. and Green, M.R. (1990) Genes Dev. 4, 2376–2382.
[19] Fujii, M., Tsuchiya, H., Chuhjo, T., Akizawa, T. and Seiki, M. (1992) Genes Dev. 6, 2066–2076.
[20] Moran, E. (1993) Curr. Opin. Genet. Dev. 3, 63–70.
[21] Meyer, M.-E., Gronemeyer, H., Turcotte, B., Bocquel, M.-T., Tasset, D. and Chambon, P. (1989) Cell 57, 433–442.
[22] Angel, P. and Karin, M. (1991) Biochim. Biophys. Acta 1072, 129–157.
[23] Jonat, C., Rahmsdorf, H.J., Park, K.-K., Cato, A.C.B., Gebel, S., Ponta, H. and Herrlich, P. (1990) Cell 62, 1189–1204.
[24] Miner, J.N., Yamamoto, K.R. (1992) Genes Dev. 6, 2491–2501.
[25] Doucas, V., Spyrou, G. and Yaniv, M. (1991) EMBO J. 10, 2237–2245.
[26] Oehler, T. and Angel, P (1992) Mol. Cell. Biol. 12, 5508–5515.
[27] Li, L., Chambard, J.-C., Karin, M. and Olson, E.N. (1992) Genes Dev. 6, 676–689.
[28] Bengal, E., Ransome, L., Scharfmann, R., Dwarki, V.J., Tapscott, S.J., Weintraub, H. and Verma, I.M. (1992) Cell 68, 507–519.
[29] McBride, K., Robitaille, S.T., Argentin, S., Nemer, M. and (1993) Mol. Cell. Biol. 13, 600–612.
[30] Klempnauer, K.-H., Arnold, H. and Biedenkapp, H. (1989) Genes Dev. 3, 1582–1589.
[31] Dubendorff, J.W., Whittaker, L.J., Eltman, J.T. and Lipsick, J.S. (1992) Genes Dev. 6, 2524–2535.
[32] Resar, L.M.S., Dolde, C., Barret, J.F. and Dang, C.V. (1993) Mol. Cell. Biol. 13, 1130–1136.
[33] Narayanan, R., Klement, J.F., Ruben, S.M., Higgins, K.A. and Rosen, C.A. (1992) Science 256, 367–370.
[34] Treisman, R. (1992) Trends Biochem. Sci. 17, 423–426.
[35] Ernst, W.H., Janknecht, R., Cahill, M.A. and Nordheim, A. (1994) submitted.
[36] Hill, C.S., Marais, R., John, S., Wynne, J., Dalton, S. and Treisman, R. (1993) Cell 73, 395–406.
[37] Janknecht, R., Ernst, W.H., Houthaeve, T. and Nordheim, A. (1993) Eur. J. Biochem. 216, 469–475.
[38] Norman, C., Runswick, M., Pollock, R. and Treisman, R. (1988) Cell 55, 989–1003.
[39] Zhu, H. and Prywes, R. (1992) Proc. Natl. Acad. Sci. USA 89, 5291–5295.
[40] Rivera, V.M., Sheng, M. and Greenberg, M.E. (1990) Genes Dev. 4, 255–268.
[41] Gius, D., Cao, X., Rauscher III, F.J., Cohen, D.R., Curran, T. and Sukhatme, V.P. (1990) Mol. Cell. Biol. 10, 4243–4255.
[42] Wilson, T. and Treisman, R. (1988) EMBO J. 7, 4193–4202.
[43] Lucibello, F.C., Lowag, C., Neuberg, M. and Müller, R. (1989) Cell 59, 999–1007.
[44] Hollingsworth, R.E.J., Hensey, C.E. and Lee, W.-H. (1993) Curr. Opin. Genet. Dev. 3, 55–62.
[45] Boncinelli, E., Simeone, A., Acampora, D. and Mavillo, F. (1991) Trends Gen. 7, 329–334.