Selective repression of transcriptional activators at a distance by the *Drosophila* Krüppel protein

(transcription/RNA polymerase II/transcription factor Sp1)

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ABSTRACT The Krüppel (Kr) protein, bound at kilobase distances from the start site of transcription, represses transcription by RNA polymerase II in mammalian cells. Repression is monotonically dependent on the dose of Kr protein and the presence of Kr binding site(s) on the DNA. These data suggest an inhibitory protein–protein interaction between the Kr protein and proximal transcription factors. Repression by Kr depends on the specific activator protein driving transcription. In particular, Kr protein selectively represses transcription mediated by the Sp1 glutamine-rich activation domain, tethered to the promoter by a GAL4 DNA-binding domain, but does not repress transcription stimulated by the acidic GAL4 activator. We believe this represents repression by a quenching interaction between DNA-bound Kr protein and the activation region of Sp1, rather than competition between Sp1 and Kr for a limiting transcriptional component. Selective, context-related repression affords an added layer of combinatorial control of gene expression by sequence-specific transcription factors.

Repression of transcription at RNA polymerase II promoters may occur by mechanisms involving either steric hindrance or inhibitory protein–protein interactions. Positioned nucleosomes can repress transcription by excluding specific DNA-binding transcriptional activators (for reviews, see refs. 1–3). Promoters can be inactivated by the displacement of a strong DNA-bound activator protein by either a weak transcriptional activator or a repressor (4–6). In addition, repression can result from the occlusion of the start site of transcription by a DNA-binding protein (7, 8). Protein–protein interactions can occur in solution to down-regulate transcription, by the formation of inactive oligomeric transcription factors (9, 10). Repression by squelching (11–17) results when an excess of an activator protein sequesters limiting components of the transcriptional machinery in the nucleoplasm, preventing these components from productively participating in transcription complexes.

Repressive protein–protein interactions also occur among transcription factors simultaneously bound to a gene. Direct repression is defined as inhibition due to interaction between the DNA-bound repressor and the basal transcriptional machinery (13, 14). By repressing basal transcription, transcription stimulated by many or all types of activators should also be inhibited. In contrast, quenching refers to specific interference by a DNA-bound factor of transcriptional activation mediated by a second protein (13, 14). Quenching might occur through an interaction between the repressor and either an activator or a coactivator essential for activator function. Since transcriptional activation domains fall into different classes (glutamine-rich, acidic, proline-rich) (18) which may activate transcription by independent pathways (15, 19, 20),

a repressor that quenches transcription would be predicted to interfere with only a subset of transcriptional activators.

Krüppel (Kr) is a *Drosophila* gap gene (21) which encodes a zinc-finger DNA-binding protein (22–24). Genetic data suggest that the Kr protein can function as a repressor of gene expression in the developing *Drosophila* embryo (25–33). By transfection experiments in mammalian cells we found that the Kr protein actively repressed transcription at a distance when bound to an RNA polymerase II promoter (34). Similar data have been obtained in cultured *Drosophila* cells (16, 35, 36). Transfer of the N-terminal portion of the Kr protein to a heterologous DNA-binding protein, the *lac* repressor, conferred upstream repression activity on the latter protein, showing that the DNA-binding and repression functions of Kr are distinct (34). We now demonstrate that the Kr protein selectively represses transcription activated by a glutamine-rich activator but not by an acidic activator. This indicates that Kr quenches transcription due to specific protein–protein interactions between Kr protein and part of the transcriptional apparatus mediating stimulation by a glutamine-rich activator, most likely the activator itself.

MATERIALS AND METHODS

Plasmids. CMV-Kr, CMV-LacI, and GAL4 expression plasmids and the Kr4-*tk*CAT reporter plasmid have been described (34, 37). Plasmids Kr4(–484) and Kr4(–1086), containing four Kr binding sites at the indicated distances 5' to the thymidine kinase gene (*tk*) transcription initiation site, were constructed by ligation of *Sau*3A1 fragments of pUC19 into Kr4-*tk*CAT digested with *Bam*HI. Kr8(+1400) was constructed by ligation of two 200-bp DNA fragments of Kr4-*tk*CAT, each containing four Kr binding sites, 3' to the chloramphenicol acetyltransferase (CAT) gene of pBLCAT2 (38). Kr4G5BCAT was constructed by insertion of four Kr binding sites 15 bp upstream of the GAL4 binding sites of pG5BCAT (15, 39). The GAL4(1–147)-Sp1 plasmid encodes the DNA-binding domain of GAL4 and contains an *Eco*RI fragment encoding the second glutamine-rich region of Sp1 (gift of M. Ptashne, Harvard University). Kr4-*tk*GH was constructed by replacing the CAT gene of Kr4-*tk*CAT with a fragment of the human growth hormone (hGH) gene from p ϕ GH (40). Wild-type *tk*GH and ψ -*tk*GH (pseudo-wild-type) plasmids (gift of P. Casaz, Dana–Farber Cancer Institute) were constructed by insertion of DNA fragments containing the wild-type or pseudo-wild-type *tk* promoter regions (41) into p ϕ GH. For the generation of RNA probes, a *Sac*I-*Eco*RI fragment of the wild-type *tk*GH gene was inserted into pBluescript SK(+) (Stratagene).

Abbreviations: CAT, chloramphenicol acetyltransferase; *tk*, thymidine kinase gene; hGH, human growth hormone.

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Cell Culture and Transfection. CV-1 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum in a 5% CO₂ environment and plated at 5×10^5 cells per 100-mm dish 24 hr before transfection. Reporter and effector plasmids were transfected by the calcium phosphate method (34, 42) in amounts indicated in the figure legends, along with pMTGH (40) as an internal control of transfection efficiency. In some experiments cells were coincubated overnight with chloroquine (50 μ M). At 48 hr posttransfection, cell medium was harvested for GH and cell lysates were assayed for CAT activity (34, 42, 43). Chromatograms were quantitated by Betascope (Betagen). Normalized CAT activity is the percent conversion of chloramphenicol to acetylated chloramphenicol divided by the level of GH (ng/ml) in the same transfection experiment.

RNase Protection. At 48 hr posttransfection, total cellular RNA was harvested using RNazol (Chimex-Biotech, Friendswood, TX). An antisense ³²P-labeled RNA probe was transcribed and purified and RNase protection was performed (44). The reporter gene yielded a cluster of transcripts of 105–120 nt in length while the internal control generated a cluster of 65- to 80-nt fragments due to the insertion of a *Bam*HI linker into the pseudo-wild-type *tk* gene. Relative levels of RNA transcripts displayed in the autoradiographs were quantitated using an LKB/Pharmacia densitometer with Ultrascan software.

RESULTS

Kr Reduces the Accumulation of mRNA. The Kr4-*tk*GH reporter containing the herpes simplex virus *tk* promoter with four upstream Kr binding sites (34) was transfected into mammalian cells, along with either the Kr expression plasmid (CMV-Kr) or a control expression vector (CMV-LacI). As an internal control, the experiment included a "pseudo-wild-type" *tk* promoter (41) lacking Kr binding sites (ψ -*tk*GH). In the presence of Kr, correctly initiated transcripts from the Kr4-*tk*GH construct, detected by RNase protection, were decreased by a factor of 3 relative to transcription from the internal control (Fig. 1). Therefore repression occurred at the level of mRNA production.

The internal control signal also decreased upon expression of Kr, most probably reflecting a reduced overall transfection efficiency in this experiment. Alternatively, the internal control may normalize for some DNA binding site-independent repression activity by Kr. In fact, we have consistently noted up to 2-fold repression of an internal control reporter plasmid (mouse metallothionein promoter-GH) at high input levels of transfected CMV-Kr (data not

FIG. 1. Kr protein represses gene expression at the RNA level. Kr4-*tk*GH (5 μ g) and ψ -*tk*GH (5 μ g) were transfected into CV-1 cells along with 10 μ g of either CMV-Kr (lane 2) or control CMV-LacI (lane 1) expression plasmid. Correctly initiated transcripts from the reporter and internal control genes detected by RNase protection assay are indicated by brackets. The CMV-Kr lane was exposed 4.7 times longer than the CMV-LacI lane, to allow for direct comparison of the internal controls.

shown). Repression independent of specific Kr DNA binding sites may be mediated by Kr interaction with low-affinity DNA binding sites, which becomes apparent at high protein levels. Such repression could also occur via specific interaction off the DNA between Kr and specific activator(s) as has been argued for repression by Kr of transcription activated by the Hunchback (Hb) protein (16). To focus on the mechanism of repression dependent on Kr binding sites in the promoter (cis-acting), our analyses conservatively underestimate repression by the Kr protein.

Dose-Dependent Repression by Kr. Site-dependent repression by Kr could be due to competition for binding to a site normally occupied by a mammalian activator protein. Alternatively, a mammalian activator interacting with Kr binding sites might be squelched by Kr. Both possibilities are unlikely, however, as insertion of Kr binding sites upstream of the *tk* promoter or upstream of the TATA box in the G5BCAT constructs described below did not lead to activation of these promoters in the absence of Kr protein (ref. 34; see Fig. 4A).

The Kr4-*tk*CAT reporter gene (34) was expressed in CV-1 cells with increasing amounts of the Kr expression vector. At transfected CMV-Kr over 200 ng, a monotonic decline in relative CAT activity was noted (Fig. 2). Low levels (20 and 200 ng) of transfected CMV-Kr did not appreciably affect the reporter gene (Fig. 2). In contrast, one laboratory observed substantial activation of transcription from two different promoters in *Drosophila* Schneider cells in the presence of low levels of Kr expression (36), whereas another laboratory, using different reporter constructs, observed only repression by Kr (16). In our experiments, Kr activated transcription in mammalian cells only under limited circumstances (see below). Whether these data indicate differences in the mechanisms of action of Kr protein in mammalian and *Drosophila* cells remains to be determined.

Kr Represses Transcription in a Distance-Independent Manner. CMV-Kr or CMV-LacI (8 μ g) was coexpressed with reporter plasmids (4 μ g) containing Kr binding sites at a distance from the start site of transcription. CAT expression from each of these plasmids was repressed to a similar extent in the presence of Kr protein. The fold repression and standard errors from 6–10 experiments were as follows: Kr4(-143), 4 ± 0.3 ; Kr(-484), 4 ± 1.0 ; Kr4(-1086), 2.7 ± 0.1 ; Kr8(+1400), 2.6 ± 0.1 . An isogenic reporter construct lacking Kr binding sites was not significantly affected by the expression of Kr (ref. 34; data not shown). Distance-independent repression rules out the possibility that Kr

FIG. 2. Dose-dependent repression by Kr protein. CV-1 cells were cotransfected with Kr4-*tk*CAT reporter (4 μ g), pMTGH (1 μ g), and the indicated amounts of CMV-Kr or CMV-LacI. Vertical axis indicates the ratio of CAT activities (normalized for transfection efficiency) obtained in the presence or absence of Kr. Error bars represent SE from 6–10 experiments.

displaces activating transcription factors from the *tk* promoter through steric hindrance and suggests a mechanism involving protein-protein interactions between Kr and proximally bound factors.

Kr Repression of a Minimal Promoter Activated by Defined Activation Domains. To identify possible molecular target(s) of Kr, we determined the ability of Kr to repress transcription from a minimal promoter, Kr4G5BCAT (see Fig. 4A), stimulated by activators containing different activation domains. The nondetectable basal expression from Kr4G5BCAT was stimulated by either wild-type GAL4 or the chimeric activator GAL4(1-147)-Sp1 (Fig. 3). In response to increasing amounts of GAL4 expression plasmid, CAT expression increased dramatically and monotonically. Activation by the GAL4(1-147)-Sp1 fusion gene was considerably less potent (10-fold, vs. >100-fold for GAL4) and saturated at 200 ng of plasmid per 100-mm dish. At even higher input levels of GAL4-Sp1 expression construct, CAT expression decreased. Electrophoretic mobility-shift assays indicated that the GAL4 DNA-binding activity in extracts of transfected cells increased approximately linearly with the amount of input plasmid (data not shown). These data suggest that the decrease in transcription seen in the presence of high levels of GAL4-Sp1 protein was due to self-squelching by the glutamine-rich activation domain of Sp1.

To determine whether Kr could repress transcription mediated by the two different activators, the amount of each activator yielding approximately half-maximal transcription of the Kr4G5BCAT reporter gene was transfected in combination with either CMV-Kr or CMV-LacI. Strikingly, transcription activated by the acidic GAL4 activation domain was not repressed by Kr, whereas transcription activated by the Sp1 activation domain was repressed (5- to 8-fold in these experiments; Fig. 4C). Kr did not repress transcription from the G5BCAT reporter gene alone, which lacks Kr binding sites, when activated by either of the GAL4 activators (Fig. 4B). This rules out the possibility that Kr indirectly represses transcription by reducing the levels of the GAL4 activator proteins.

Since the GAL4-Sp1 activator was considerably less potent than the GAL4 activator (Fig. 3), we wished to establish that the ability of Kr to repress only GAL4-Sp1-activated transcription was due to the nature of the activating protein. CV-1 cells were transfected with a fixed amount of either CMV-Kr or CMV-LacI, a fixed amount of Kr4G5BCAT reporter plasmid, and increasing amounts of GAL4 or

FIG. 3. Activation of the Kr4G5BCAT reporter gene by GAL4 (●) and GAL4-Sp1 (○). CV-1 cells were cotransfected with Kr4G5BCAT (2 μg), pMTGH (1 μg), and the indicated amounts of GAL4 or GAL4(1-147)-Sp1 effector plasmids. Vertical axis represents the relative CAT activity, normalized to hGH expression.

FIG. 4. Selective, DNA binding site-dependent repression of transcription by Kr. (A) CV-1 cells were cotransfected with Kr4G5BCAT (Right) or G5BCAT (Left) (2 μg), pMTGH (0.5 μg), and either no expression plasmid, GAL4(1-147)-Sp1 (0.1 μg), or GAL4 expressor plasmid (0.1 μg). Average normalized CAT activities $\pm$ SE from four to six independent experiments are displayed above a representative chromatogram. (B) CV-1 cells were cotransfected with the G5BCAT reporter plasmid (2 μg), pMTGH (1 μg), either the GAL4(1-147)-Sp1 expressor plasmid (0.2 μg) or the GAL4 expressor plasmid (0.1 μg), and either CMV-Kr or CMV-LacI as indicated. (C) CV-1 cells were cotransfected with Kr4G5BCAT (2 μg), pMTGH (1 μg), either the GAL4 (0.1 μg) or GAL4(1-147)-Sp1 (0.2 μg) expressor plasmid, and either CMV-Kr or CMV-LacI.

GAL4(1-147)-Sp1 activator plasmids (Fig. 5). This allowed Kr function to be examined at similar absolute levels of transcription, mediated by the two different activators. At 20 ng of transfected GAL4 or GAL4(1-147)-Sp1 expression plasmid, similar levels of trans-activation were observed (Fig. 3). At these levels of GAL4(1-147)-Sp1 expression plasmid, Kr repressed CAT expression (average 2.3-fold in this set of experiments; Fig. 5A). At higher levels of GAL4(1-147)-Sp1 activator plasmid that saturate transcriptional activation (>1 μg; Fig. 3), transcriptional repression was lost.

In sharp contrast, Kr never repressed transcription mediated by the acidic GAL4 protein. In fact, at low levels of GAL4 activator plasmid, CAT expression was higher in the presence of Kr. As the amount of GAL4 activator was increased, the ratio of reporter gene expression in the presence versus absence of Kr dropped to one, indicating no effect of Kr.

DISCUSSION

Selective Repression by Kr. Our data indicate that Kr protein selectively interferes with the pathway by which

FIG. 5. (A) Repression by Kr can be overcome by increasing expression of GAL4-Sp1. CV-1 cells were cotransfected with Kr4G5BCAT (2 μ g), pMTGH (1 μ g), either CMV-Kr or CMV-LacI (10 μ g), and the indicated amounts of GAL4(1-147)-Sp1 expressor plasmid. Vertical axis indicates fold change in normalized CAT activities obtained in the presence of Kr. Error bars represent SE from 6–10 experiments. Typical values for the percentage of chloramphenicol acetylated by extracts transfected with increasing levels of GAL4(1-147)-Sp1 in the presence of Kr are 0.3% (20 ng), 0.6% (200 ng), 1.8% (2 μ g), and 1.5% (6.6 μ g). (B) Kr activates transcription in the presence of low levels of GAL4 acidic activator. CV-1 cells were cotransfected with Kr4G5BCAT (2 μ g), pMTGH (1 μ g), either CMV-Kr or CMV-LacI (10 μ g), and the indicated amounts of GAL4 expressor plasmid. Typical values for the percentage of chloramphenicol acetylated by extracts transfected with increasing levels of GAL4 in the presence of Kr are 7.6% (20 ng), 15% (200 ng), 27% (2 μ g), and 86% (6.6 μ g). Axes and error bars are as indicated above.

glutamine-rich activators such as Sp1 stimulate transcription. This might occur by a protein–protein interaction between Kr and Sp1, with the repression region of Kr directly quenching (13, 14) the activation function of Sp1. With increasing intracellular levels of GAL4-Sp1 activator, and presumably greater occupancy on the DNA template, this quenching could be overcome. Alternatively, the Kr protein and Sp1 might compete for binding to a coactivator or TFIID-associated factor (TAF) (45–51) capable of mediating transcriptional activation by Sp1, with binding of Kr to the coactivator being nonproductive. However, we believe that our data argue against Kr interaction with a coactivator. If Kr bound the same coactivator as Sp1, one would expect that self-squelching by Sp1 would be observed at lower input levels of GAL4-Sp1 expression plasmid in the presence of Kr than in its absence and that self-squelching at high levels of Sp1 plasmid would be enhanced in the presence of Kr. Instead, the level of GAL4(1-147)-Sp1 plasmid at which self-squelching occurred was identical in the presence or absence of Kr (data not shown). In addition, at the highest levels of transfected GAL4(1-147)-Sp1, CAT expression was higher in the presence of Kr (Fig. 5A). This would be expected if Kr bound to the Sp1 activation domain and reduced the self-squelching by GAL4(1-147)-Sp1. Therefore our data are more consistent with a quenching interaction,

due to a direct protein–protein interaction between the DNA-bound Kr protein and the glutamine-rich Sp1 activation domain. The repression region within the N-terminal portion of the Kr protein includes a predicted α -helix with one face consisting of multiple glutamine residues (J.D.L. and U.H., unpublished work) which might interact with the glutamine-rich region of Sp1, preventing its productive interaction with other components of the transcriptional machinery.

Since Kr quenches transcription mediated by a specific activator rather than all activators, it is unlikely that Kr blocks a step required for basal transcription of all promoters such as the binding of the TATA-binding protein (TBP), a factor found in the multiprotein TFIID complex. However, selective repression could result from an interaction between Kr and a specific portion of TBP. Although unlikely, selective repression does not entirely rule out an interaction between Kr and other basal transcription factors, as recent data indicate that some “general” transcription factors may be preferentially used on particular promoters (52). The full elucidation of the molecular mechanism of Kr repression will require biochemical studies of possible interactions between Kr and Sp1, TBP, or candidate coactivators such as the TBP-associated factors (TAFs) (50, 51, 53).

The selective, quenching mode of repression by Kr contrasts sharply with the mechanism of repression of the *Drosophila* even-skipped (*eve*) protein. *In vitro* studies indicated that *eve* protein repressed basal transcription, most likely by destabilizing the preinitiation complex (54). *In vivo* studies showed that *eve* could repress transcription activated by a number of different activator proteins, also consistent with a direct mechanism for repression by interaction with the basal transcriptional machinery (13, 55).

The ability of Kr to interfere with Sp1 function may be relevant to *Drosophila* biology. Although *Drosophila* cells do not contain Sp1, a number of *Drosophila* transcriptional activators do contain glutamine-rich regions, and a glutamine-rich region of the Antennapedia protein can substitute for the natural activation region of Sp1 (56).

Distance-Independent Repression. Distance-independent repression rules out the possibility that Kr is simply sterically blocking the *tk* promoter. In addition, it is unlikely that Kr, when bound at great distances from the promoter, could precisely position a nucleosome (57) over the *tk* promoter and occlude its access to transcription factors. Repression at kilobase distances supports a mechanism requiring protein–protein interactions between distantly bound Kr protein and proximal factors, such as Sp1, looping out intervening DNA (58).

We observed 3- to 12-fold transcriptional repression by Kr protein using synthetic promoter constructs (data herein and ref. 34). In contrast, Kr strongly (≈ 30 -fold) repressed a portion of the engrailed promoter containing overlapping homeodomain and Kr binding sites and activated by the z2 homeodomain protein (16). In this case, Kr could both displace the z2 activator and quench transcriptional activation by residual factors bound to the engrailed promoter (13). In HeLa or CV-1 cells, our data indicate that Kr protein does not displace an endogenous activator, leaving quenching of activation directed by the Sp1 protein bound to the *tk* promoter as the sole mechanism of repression.

Transcriptional Activation by Kr. Kr protein can also activate transcription (24, 27, 36). At low input levels of GAL4 effector plasmid driving transcription of Kr4G5BCAT, we found that Kr reproducibly activated transcription (Fig. 5B). We have not determined whether the same region of the Kr protein is required for activation in mammalian cells and in *Drosophila* Schneider cells (36). Transcriptional activation by Kr might occur through an indirect mechanism. If the acidic and glutamine-rich transcriptional machineries competed for interaction with a common, limiting factor, and Kr

were to block factors of the glutamine activation-dependent pathway from interacting with the common factor, the alternative, acidic activator pathway would be boosted. Alternatively, Kr may simply be a weak transcriptional activator in mammalian cells, with its repressive effect predominating in the test systems we employed. Hence the transcriptional function of Kr might be highly context-dependent.

Selective Kr Repression and *Drosophila* Development. The ability of Kr to repress at a distance must be reconciled with the independent action of the cis-acting elements controlling the spatial expression of *Drosophila* pair-rule genes such as *eve*. In particular, Kr bound to the stripe 2 element of the *eve* promoter does not repress transcription directed by the stripe 3 *eve* element, which is devoid of Kr binding sites (22) and located about 1.5 kb further upstream (26, 28). These data can be reconciled by either of two models. Cis-acting elements controlling the spatial expression of *Drosophila* segmentation genes may be functionally separated into distinct transcriptional domains, influenced by particular transcription factors only in certain regions of the embryo (59). Alternatively, "short-range" repression by Kr of the stripe 2 element but not the stripe 3 element in the *eve* promoter may be due to the overlap of activator sites and Kr repressor binding sites in the stripe 2 element (22, 28, 29) and to the nature of the activator proteins binding to each element (glutamine-rich vs. acidic). Hb activator binding sites are found in both the stripe 2 and 3 elements (22, 23) and bicoid binding sites in the stripe 2 element (28). Hb contains both acidic and glutamine-rich sequences (60). If activation of *eve* stripe 3 depended upon acidic regions in Hb or another acidic activator and activation of stripe 2 depended on the glutamine-rich regions of Hb or bicoid, Kr protein bound to the stripe 2 element might be unable to repress the acidic activation of stripe 3. Selective repression by Kr could, therefore, play a role in *Drosophila* physiology and represent an additional combinatorial level of control of gene expression.

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- Svaren, J. & Chalkley, R. (1990) *Trends Genet.* 6, 52–56.
- Felsenfeld, G. (1992) *Nature (London)* 355, 219–224.
- Grunstein, M. (1990) *Trends Genet.* 6, 401–405.
- Strömstedt, P.-E., Poellinger, L., Gustafsson, J.-Å. & Carlstedt-Duke, J. (1991) *Mol. Cell. Biol.* 11, 3379–3383.
- Superti-Furga, G., Barberis, A., Schaffner, G. & Busslinger, M. (1988) *EMBO J.* 7, 3099–3107.
- Akerblom, I. E., Slater, E. P., Beato, M., Baxter, J. D. & Mellon, P. M. (1988) *Science* 241, 350–353.
- Rio, D., Robbins, A., Myers, R. & Tjian, R. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5706–5710.
- Hansen, U., Tenen, D. G., Livingston, D. M. & Sharp, P. A. (1981) *Cell* 27, 603–612.
- Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L. & Weintraub, H. (1990) *Cell* 61, 49–59.
- Jones, N. (1990) *Cell* 61, 9–11.
- Gill, G. & Ptashne, M. (1988) *Nature (London)* 334, 721–724.
- Ptashne, M. (1988) *Nature (London)* 335, 683–689.
- Levine, M. & Manley, J. L. (1989) *Cell* 59, 405–408.
- Renkawitz, R. (1990) *Trends Genet.* 6, 192–197.
- Martin, K. J., Lillie, J. W. & Green, M. R. (1990) *Nature (London)* 346, 147–152.
- Zuo, P., Stanojević, D., Colgan, J., Han, K., Levine, M. & Manley, J. L. (1991) *Genes Dev.* 5, 254–264.
- Xiao, J. H., Davidson, I., Matthes, H., Garnier, J.-M. & Chambon, P. (1991) *Cell* 65, 551–568.
- Mitchell, P. J. & Tjian, R. (1989) *Science* 245, 371–378.
- Tasset, D., Tora, L., Fromental, C., Scheer, E. & Chambon, P. (1990) *Cell* 62, 1177–1187.
- Berger, S. L., Piña, B., Silverman, N., Marcus, G. A., Agapite, J., Regier, J. L., Triezenberg, S. J. & Guarente, L. (1992) *Cell* 70, 251–265.
- Gaul, U. & Jäckle, H. (1987) *Trends Genet.* 3, 127–131.
- Stanojević, D., Hoey, T. & Levine, M. (1989) *Nature (London)* 341, 331–335.
- Treisman, J. & Desplan, C. (1989) *Nature (London)* 341, 335–337.
- Pankratz, M. J., Hoch, M., Seifert, E. & Jäckle, H. (1989) *Nature (London)* 341, 337–339.
- Gaul, U. & Jäckle, H. (1989) *Development* 107, 651–662.
- Goto, T., Macdonald, P. & Maniatis, T. (1989) *Cell* 57, 413–422.
- Pankratz, M. J., Seifert, E., Gerwin, N., Billi, B., Nauber, U. & Jäckle, H. (1990) *Cell* 61, 309–317.
- Stanojević, D., Small, S. & Levine, M. (1991) *Science* 254, 1385–1387.
- Ingham, P. W., Ish-Horowicz, D. & Howard, K. R. (1986) *EMBO J.* 5, 1659–1665.
- Carroll, S. B. & Vavra, S. H. (1989) *Development* 107, 673–683.
- Frasch, M. & Levine, M. (1987) *Genes Dev.* 1, 981–995.
- Mohler, J., Eldon, E. D. & Pirrotta, V. (1989) *EMBO J.* 8, 1539–1548.
- Rothe, M., Nauber, U. & Jäckle, H. (1989) *EMBO J.* 8, 3087–3094.
- Licht, J. D., Grossel, M. J., Figge, J. & Hansen, U. M. (1990) *Nature (London)* 346, 76–79.
- Small, S., Kraut, R., Hoey, T., Warrior, R. & Levine, M. (1991) *Genes Dev.* 5, 827–839.
- Sauer, F. & Jäckle, H. (1991) *Nature (London)* 353, 563–566.
- Kakidani, H. & Ptashne, M. (1988) *Cell* 52, 161–167.
- Luckow, B. & Schütz, G. (1987) *Nucleic Acids Res.* 15, 5490.
- Carey, M., Lin, Y.-S., Green, M. R. & Ptashne, M. (1990) *Nature (London)* 345, 361–364.
- Selden, R. F., Howie, K. B., Rowe, M. E., Goodman, H. M. & Moore, D. D. (1986) *Mol. Cell. Biol.* 6, 3173–3179.
- McKnight, S. L. & Kingsbury, R. (1982) *Science* 217, 316–324.
- Kingston, R. E. (1987) in *Current Protocols in Molecular Biology*, eds. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (Greene and Wiley, New York), pp. 911–919.
- Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* 2, 1044–1051.
- Gilman, M. (1987) in *Current Protocols in Molecular Biology*, eds. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (Greene and Wiley, New York), pp. 471–478.
- Flanagan, P. M., Kelleher, R. J., III, Sayre, M. H., Tschochner, H. & Kornberg, R. D. (1991) *Nature (London)* 350, 436–438.
- Pugh, B. F. & Tjian, R. (1990) *Cell* 61, 1187–1197.
- Berger, S. L., Cress, W. D., Cress, A., Triezenberg, S. J. & Guarente, L. (1990) *Cell* 61, 1199–1208.
- Kelleher, R. J., III, Flanagan, P. M. & Kornberg, R. D. (1990) *Cell* 61, 1209–1215.
- Ptashne, M. & Gann, A. A. F. (1990) *Nature (London)* 346, 329–331.
- Hoey, T., Weinzerl, R. O. J., Gill, G., Chen, J.-L., Dynlacht, B. D. & Tjian, R. (1993) *Cell* 72, 247–260.
- Meisterernst, M., Roy, A. L., Lieu, H. M. & Roeder, R. G. (1991) *Cell* 66, 981–993.
- Parvin, J. D., Timmers, H. T. M. & Sharp, P. A. (1992) *Cell* 68, 1135–1144.
- Timmers, H. T. M. & Sharp, P. A. (1991) *Genes Dev.* 5, 1946–1956.
- Johnson, F. B. & Krasnow, M. A. (1992) *Genes Dev.* 6, 2177–2189.
- Han, K. & Manley, J. L. (1993) *Genes Dev.* 7, 491–503.
- Courey, A. J. & Tjian, R. (1988) *Cell* 55, 887–898.
- Roth, S. Y., Dean, A. & Simpson, R. T. (1990) *Mol. Cell. Biol.* 10, 2247–2260.
- Ptashne, M. (1986) *Nature (London)* 322, 697–701.
- Peifer, M., Karch, F. & Bender, W. (1987) *Genes Dev.* 1, 891–898.
- Tautz, D., Lehman, R., Schnürch, H., Schuh, R., Seifert, E., Kienlin, A., Jones, K. & Jäckle, H. (1987) *Nature (London)* 327, 383–389.