3043, segregating 3:1 on medium containing phosphinothricin, was used in further experiments.
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12. The coding region of the *cre* gene (*25*) was fused translationally to the *virE2* and *virF* gene of pTi15955, respectively, under control of the respective *vir* promoter regions. Both NH_2-terminal and COOH-terminal fusions were made. The fusions with *virF* contained an additional NH_2-terminally located nuclear localization signal (NLS) from simian virus SV40 (*NLS::cre::virF* and *NLS::virF::cre*, respectively). The *virE2* fusions (*cre::virE2* and *virE2::cre*) were coordinately expressed with *virE1* under control of the *virE* promoter. As a control, the *cre* gene was linked directly to the *virE* promoter, at the start codon position of *virE2*. The fusion genes were cloned into broad host range, nonmobilizable plasmid pRL662. This plasmid was the result of replacing the kanamycin resistance gene and the *mob* region from pBBR1 MCS2 (*26*) by a gentamycin resistance marker.
13. A. C. Vergunst *et al.*, data not shown.
14. Roots from 10-day-old seedlings homozygous for excision locus 3043 (T3 or T4) were cocultivated for 2 days with bacteria. The explants were transferred to shoot induction medium containing kanamycin (50 mg/liter) and timentin (100 mg/liter). The number of kanamycin-resistant calli was estimated 3 weeks after cocultivation. Control cocultivations of LBA2561 (*NLS::cre::virFΔ42N*) and LBA1149 (*cre::virE2*) with wild-type *Arabidopsis* C24 root explants did not result in kanamycin-resistant calli.
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17. Chromosomal DNA was isolated from Km^r shoots (*11*). PCR reactions with primers a (5′-GAACTCGCCGTAAAGACTGGCG) and b (5′-GCGCTGACAGCCGGAACACG) were performed as follows: hot start for 5 min of 96°C, followed by 35 cycles of 1 min of 94°C, 2 min of 57°C, and 2 min of 72°C, with Ex-Taq polymerase (BioWhittaker, Belgium) according to the manufacturer.
18. A fusion was made, in which 126 5′ base pairs of *virF* were deleted, resulting in fusion protein NLS::Cre::VirFΔ42N. Deletion of the 5′ 498 base pairs of *virF* and translational fusion to *cre* resulted in fusion protein NLS::Cre::VirFΔ166N.
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A Basal Transcription Factor That Activates or Represses Transcription

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We have identified an activity that is required for transcription of downstream promoter element (DPE)–containing core promoters in vitro. The purified factor was found to be the *Drosophila* homolog of the transcriptional repressor known as NC2 or Dr1-Drap1. Purified recombinant dNC2 activates DPE-driven promoters and represses TATA-driven promoters. A mutant version of dNC2 can activate DPE promoters but is unable to repress TATA promoters. Thus, the activation and repression functions are distinct. These studies reveal that NC2 (Dr1-Drap1) is a bifunctional basal transcription factor that differentially regulates gene transcription through DPE or TATA box motifs.

The control of transcription by RNA polymerase II involves sequence-specific DNA binding proteins that interact with cis-acting regulatory elements, numerous coactivators and corepressors, and the structure and constitution of the chromatin template. It is important to consider, however, that the eventual target of many sequence-specific DNA binding factors and coregulators is the central machinery that mediates the basal transcription process at the core promoter. Hence, there is considerable potential for the regulation of gene activity through the basal transcription process and the core promoter.

The core promoter is generally defined as the minimal set of DNA sequences (typically about 40 base pairs) that is sufficient to direct the accurate initiation of transcription by RNA polymerase II (RNAP II) and the basal factors (*1*). There are several known core promoter motifs, which include the TATA box, the TFIIB recognition element (BRE), the initiator (Inr), and the downstream promoter element (DPE). The TATA box is an A/T-rich sequence that is located about 30 nucleotides upstream of the transcription start site, and it is bound by the TATA binding protein (TBP) subunit of TFIID. The BRE is located immediately upstream of the TATA box of some TATA-containing promoters, and increases the affinity of TFIIB for the core promoter (*2*). The Inr is a conserved sequence that encompasses the transcription start site, which functions to direct accurate transcription initiation either by itself or in conjunction with a TATA or DPE motif (*3*).

The DPE is a downstream core promoter element that is located about 30 nucleotides downstream of the transcription start site (*4–6*). The DPE is conserved from *Drosophila* to humans, and appears to be as common as the TATA box in *Drosophila* core promoters (*6*). A typical DPE-containing promoter has Inr and DPE motifs and lacks a TATA box. TFIID binds to the Inr and DPE motifs through its TAF (TBP-associated factor) subunits. The addition of a DPE in its downstream position can compensate for the loss of transcription activity that occurs upon mutation of an upstream TATA box. Thus, the activity of core promoters can be dependent on the TATA box (TATA-driven promoters) or the DPE motif (DPE-driven promoters).

We observed that high-salt nuclear extracts contain a DPE-specific activator that is deficient in low-salt nuclear extracts (Fig. 1A) (*7*). Therefore, we developed a two-template assay for the purification of this DPE-specific transcription factor (DSTF). The assay involves the simultaneous use of a DPE-driven core promoter (*Drosophila jockey*) and a TATA-driven core promoter [*Drosophila hunchback* promoter 2 (*hb*P2)] in conjunction with the low-salt nuclear extract (*8*). The presence of DSTF complements the deficiency in the low-salt nuclear extract and activates the DPE-driven promoter but not the TATA-driven promoter (Fig. 1B). In fact, the activation of the DPE-driven promoter by DSTF is accompanied by repression of the TATA-driven promoter (Fig. 1B) (*9*). By using this assay, we purified DSTF activity from the high-salt nuclear extract through seven chromatographic steps (*8*) (Fig. 1C). This purification yielded two polypeptides with apparent molecular masses of 43 kD and 22 kD that copurified with DSTF activity (Fig. 1D).

Protein microsequencing of the two DSTF

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polypeptides and subsequent database analysis (*8*) revealed that the 43-kD subunit is homologous to *Saccharomyces cerevisiae* Bur6 (*10*) and human Drap1/NC2α (*11*, *12*), and that the 22-kD subunit is homologous to human Dr1/NC2β (*8*, *12–14*). These findings indicate that DSTF is the *Drosophila* homolog of the transcriptional repressor known as NC2 or Dr1-Drap1 (*11–13*, *15*). We therefore renamed DSTF as dNC2, for *Drosophila* NC2.

NC2 (Dr1-Drap1) was identified in biochemical studies as a transcriptional repressor of TATA-driven promoters [for a review, see (*16*)]. It consists of two subunits termed Bur6/Drap1/NC2α (larger subunit) and Dr1/NC2β (smaller subunit), and it binds directly to the TBP subunit of the TFIID complex (*11–13*, *15*). The extent of repression by NC2 was found to vary with different core promoters (*17*). Activation of basal transcription by NC2 in vitro has not been observed, except in one instance in which NC2 was found to effect a twofold increase in transcription (from the TATA-driven adenovirus major late promoter) by counteracting a twofold repression by TFIIA (*17*). Genetic analyses of NC2 in *S. cerevisiae* have provided strong evidence that NC2 is a global repressor of transcription by RNAP II (*10*, *18*). It is notable, however, that a *bur6-1* mutant strain (Bur6 is the large subunit of yeast NC2) was observed to exhibit defects in transcriptional activation in vivo, although it is not known whether these positive effects of yeast NC2 are direct or indirect (*10*).

dNC2 subunits (dNC2α and dNC2β) are associated in a crude *Drosophila* embryo extract, as shown by reciprocal coimmunoprecipitation experiments with antibodies to a dNC2α peptide (anti-dNC2α peptide) and antibodies to full-length recombinant dNC2β (anti-dNC2β) (Fig. 2A) (*8*). To test whether dNC2 is responsible for DSTF activity (i.e., the ability to activate transcription from DPE-driven promoters), we immunoprecipitated dNC2 from a high-salt nuclear extract with the anti-dNC2α peptide antibodies, eluted the dNC2 from the immunoprecipitate with the dNC2α peptide, and performed in vitro transcription reactions with the resulting eluate (*8*). The dNC2α peptide is able to elute DSTF activity from an anti-dNC2α peptide immunoprecipitate, but not from a control anti-dNC2β immunoprecipitate (Fig. 2B). This experiment provides evidence that native dNC2 mediates the activation of DPE-driven promoters.

To test the transcriptional activity of dNC2, we cosynthesized dNC2α and dNC2β in *Escherichia coli* and purified the recombinant dNC2 to near homogeneity (Fig. 3A) (*8*). In vitro transcription analyses revealed that the purified recombinant dNC2 is a general activator of DPE-driven core promoters (Fig. 3B, upper panel). With three different TATA-driven core promoters, we observed a variable extent of repression by recombinant dNC2 (Fig. 3B, lower panel). In addition, we observed that the DPE-specific activation function as well as the TATA-specific repression function of dNC2 requires both of its subunits (*8*).

Does dNC2 have a single function that mediates both TATA repression and DPE activation, or does it have separate functions for repression and activation? To investigate this question, we purified and analyzed two COOH-terminally truncated versions of dNC2 (Fig. 3A) (*8*). dNC2β(1-108) and dNC2β(1-91) lack portions of the protein that are homologous to regions of human Dr1/NC2β which possess transcriptional repression and TBP binding activity (*12*, *19*, *20*). Both mutant proteins possess the "histone-fold" multimerization motifs. The resulting heteromeric dNC2α–dNC2β(1-108) and dNC2α–dNC2β(1-91) proteins can function

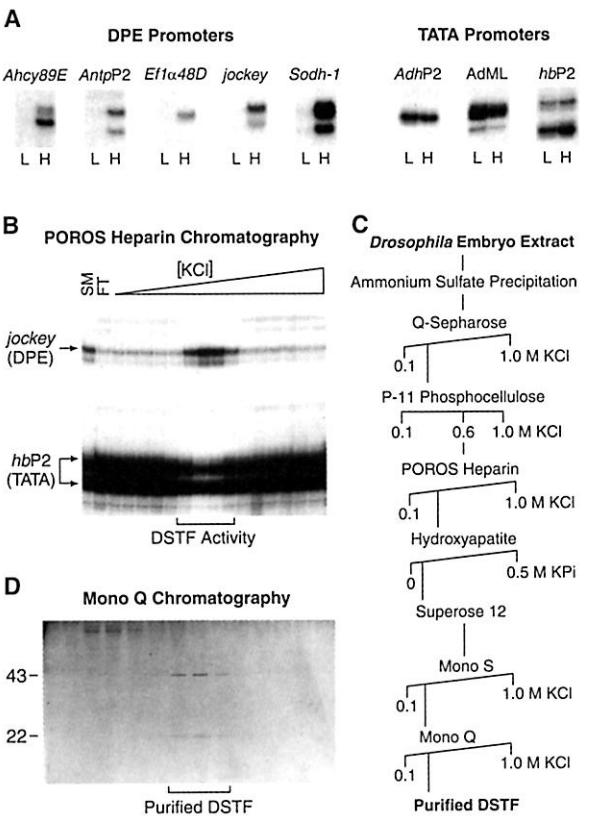

Fig. 1. Purification of the DPE-specific transcription factor, DSTF. **(A)** Identification of a DPE-specific transcription activity. The indicated DPE- or TATA-driven promoters (250 ng each template DNA) were subjected to in vitro transcription and primer extension analysis with either a low-salt (L) or a high-salt (H) nuclear extract (*8*). **(B)** Two-template DSTF activity assay with POROS heparin chromatography fractions. In vitro transcription reactions were performed with the low-salt nuclear extract (*8*) and contained 250 ng of a DPE-driven template (*jockey*) and 25 ng of a TATA-driven template (*hbP2*). SM, starting material; FT, column flowthrough. **(C)** Scheme for the purification of DSTF (*8*). **(D)** Two polypeptides copurify with DSTF activity. Mono Q chromatography fractions were subjected to 12.5% SDS–polyacrylamide gel electrophoresis (SDS-PAGE), and proteins were visualized by silver staining. The apparent molecular masses (in kD) of the polypeptides are indicated on the left.

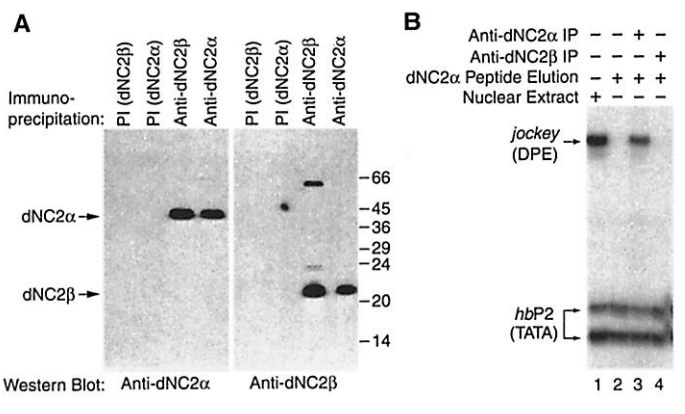

Fig. 2. DSTF is the *Drosophila* homolog of NC2 (Dr1-Drap1). **(A)** Coimmunoprecipitation of dNC2α and dNC2β from a crude extract derived from *Drosophila* embryos. Immunoprecipitations were carried out with affinity-purified antibodies to a dNC2α peptide (*8*) or full-length recombinant dNC2β [or the corresponding preimmune sera (PI)]. Western blots were performed with polyclonal antibodies to recombinant full-length dNC2α or dNC2β. **(B)** Immunoprecipitation of DSTF activity with antibodies to dNC2α. Proteins were immunoprecipitated from high salt nuclear extracts either with anti-dNC2α peptide antibody (lane 3) or with anti-dNC2β (lane 4) and were subsequently eluted with the dNC2α peptide (*8*). The eluates, as well as control reactions containing high salt extract (lane 1) or dNC2α peptide only (lane 2), were subjected to in vitro transcription analysis as in Fig. 1B.

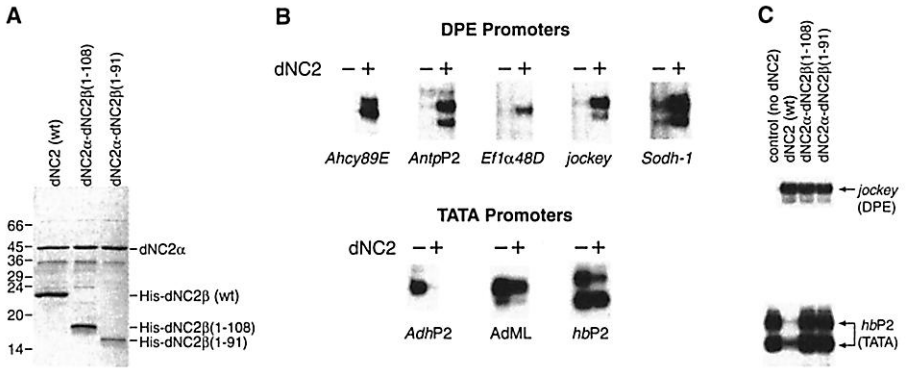

Fig. 3. Purified, recombinant dNC2 activates transcription of DPE-driven promoters and represses transcription of TATA-driven promoters. (**A**) Purification of *E. coli*–synthesized dNC2 proteins. The indicated proteins were subjected to 12% (SDS-PAGE) and staining with Coomassie Brilliant Blue R-250. The apparent molecular masses (in kD) of the polypeptides are indicated on the left. His, His_6-tagged; wt, wild type. (**B**) Transcriptional activity of recombinant wild-type dNC2. The purified protein was subjected to in vitro transcription and primer extension analysis (*8*) with DPE- or TATA-driven core promoters (250 ng of each template), as indicated. (**C**) Mutant dNC2 proteins lacking the COOH-terminal region of the dNC2β/Dr1 subunit can activate DPE promoters, but are unable to repress TATA promoters. The purified proteins were subjected to in vitro transcription analysis as described in Fig. 1B.

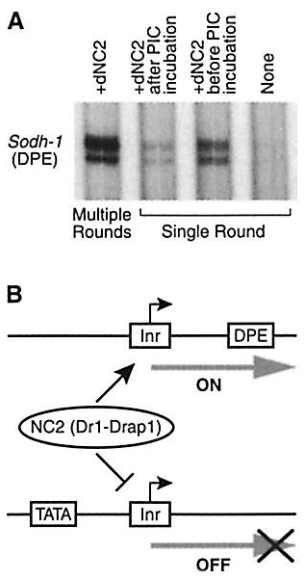

Fig. 4. dNC2 functions during the assembly of the transcription PIC. (**A**) Single-round transcription reactions (*8*) were performed with the DPE-driven *Sodh-1* core promoter (250 ng). Purified *E. coli*–synthesized dNC2 was added either before the incubation for PIC assembly (+dNC2 before PIC incubation) or after PIC assembly but 10 s before the addition of rNTPs and transcription initiation (+dNC2 after PIC incubation). In a separate reaction, buffer only was added as a control (None). For reference, a reaction in which transcription was not limited to a single round is also shown (Multiple Rounds). (**B**) NC2 (Dr1-Drap1) is a bifunctional, core promoter–specific basal transcription factor.

in the activation of DPE promoters, but they are defective for repression of TATA promoters (Fig. 3C). Thus, the activation and repression functions of dNC2 are distinct.

To determine the step at which dNC2 functions to activate transcription from DPE-driven promoters, we performed parallel single-round transcription reactions (*8*) in which purified recombinant dNC2 was added to the reaction medium either before the preinitiation complex (PIC) incubation period or after the PIC incubation period but immediately before the initiation of transcription by the addition of ribonucleoside 5′-triphosphatases (rNTPs). These experiments revealed that dNC2 activates DPE-driven transcription only when it is added to the reaction medium before PIC assembly (Fig. 4A). These results indicate that dNC2 functions at the time of PIC assembly.

Here we have found that NC2 is a bifunctional basal transcription factor that has distinct activation and repression functions at different types of core promoters (Fig. 4B). dNC2 acts at the time of PIC assembly, which suggests that dNC2 is either a component of the PIC or is required for the assembly of the PIC. The mutational analysis of the dNC2β/Dr1 subunit further revealed that the activation and repression functions are distinct. NC2 has also been found to associate with the COOH-terminal domain (CTD)–hyperphosphorylated form of RNA polymerase II (RNAP IIO) (*21*), and such NC2–RNAP IIO interactions may be involved in the activation of DPE-driven transcription by NC2. Moreover, chromatin immunoprecipitation and DNA microarray analyses with yeast NC2 (specifically, Bur6) reveal that the association of NC2 with promoters correlates with transcriptional activity and that the number of genes activated by NC2 is approximately equivalent to the number of genes that are repressed by NC2 (*22*).

In summary, the discovery that NC2 functions to activate transcription from DPE-driven core promoters indicates that there are sharp differences in the fundamental mechanisms of basal transcription from DPE- versus TATA-driven promoters. Moreover, the ability of NC2 to discriminate between these different types of core promoters suggests that NC2 could be an important target of regulatory factors (such as sequence-specific DNA binding factors or coactivators) for recruitment to core promoters. In this manner, the effective local concentration of NC2 at the core promoter could vary whereas the overall cellular concentration of NC2 could remain relatively constant. In the future, it will be interesting to identify the factors and signals that give this bifunctional transcription factor the appropriate cues to mediate the regulation of gene activity.

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