richia coli RNA polymerase (*9*).

Cotranscriptional events such as 5′ ^{m7}G capping, 3′ cleavage–polyadenylation, and pre-mRNA splicing are specific for RNAs synthesized by RNA polymerase II (*14*). In the case of capping, the presence of a 5′ triphosphate or diphosphate RNA terminus is all that is needed in vitro to permit cap formation by cellular RNA guanylyltransferase (*15*). Such termini are not restricted to pre-mRNAs, yet only polymerase II transcripts are capped with the standard ^{m7}GpppN structure. To account for this specificity, it is conceivable that cellular capping enzyme interacts specifically with RNA polymerase II or some other component of the mRNA transcription apparatus. The results in the vaccinia system provide evidence for such a binary interaction.

The timing of the various nuclear cotranscriptional mRNA processing events is not clear. It has been suggested that RNA polymerase II products synthesized in vitro in nuclear extracts are uncapped when shorter than 20 nt, but they are capped when they are 79 nt long (*16*). The refinement of the timing of capping in the vaccinia system and the interpretation that the failure to cap shorter transcripts is caused by sequestration within the RNA polymerase may be applicable to cellular transcription, as these observations are consistent with recent studies of the RNA polymerase II–elongation complex. RNase footprinting of calf thymus RNA polymerase II ternary complex paused at a unique site on a synthetic DNA template suggests that the polymerase has an RNA binding domain sufficient to protect 24 nt extending from the 3′ terminus of the paused transcript (*12*).

We conclude that acquisition of the cap structure is the earliest modification event in mRNA biogenesis. There is evidence that the presence of the cap may target the nascent transcript for splicing and polyadenylation and even for mRNA transport (*14, 18*), in which case the timely interaction of capping enzyme with the transcript would be crucial (if not actually sufficient) to establish mRNA identity.

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Spatial Control of the Gap Gene *knirps* in the *Drosophila* Embryo by Posterior Morphogen System

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The gap genes of *Drosophila* are the first zygotic genes to respond to the maternal positional signals and establish the body pattern along the anterior-posterior axis. The gap gene *knirps*, required for patterning in the posterior region of the embryo, can be activated throughout the wild-type embryo and is normally repressed from the anterior and posterior sides. These results provide direct molecular evidence that the posterior morphogen system interacts in a fundamentally different manner than do *hunchback* and *bicoid*, which are responsible for anterior pattern formation.

IN *DROSOPHILA MELANOGASTER* DEVELOPMENT, determination of polarity along the longitudinal axis is carried out by the maternal genes (*1*). The anterior determinant is *bicoid* (*bcd*), whose gene product is localized at the anterior pole of the egg (*2*). The posterior determinant is *nanos* (*nos*), and its gene product is localized at the posterior pole (*3*). The polarized distribution of these components ultimately leads to the regional expression of the zygotic gap genes (*4*). Genetic studies indicate that the anterior gap gene *hunchback* (*hb*) and the posterior gap gene *knirps* (*kni*) are key zygotic targets of *bcd* and *nos*, respectively (*4, 5*). Despite these formal similarities, the mode of action of the two maternal systems is fundamentally different. In the anterior, *bcd* directly activates and sets the expression border of *hb* (*6*). In the posterior, by contrast, *nos* does not seem to interact directly with *kni* but functions by eliminating a factor whose absence apparently allows *kni* to be expressed (*7*). This unexpected result then raised the question of how *kni* becomes initially expressed and how its spatial borders are established.

The gap gene *kni* is required for abdominal segmentation of the embryo (*5, 8*). Consistent with this function, *kni* is expressed in a posterior region of the blastoderm embryo that will give rise to the abdomen (*9*). A second expression domain is found in the anterior tip, but the function of this domain is not known and its regulation will not be discussed in this paper. We have shown that a 4.4-kb upstream sequence of *kni* directs the expression of a reporter gene in a region corresponding to the endogenous *kni* transcripts (*10*). To investigate how *kni* was spatially regulated, we made various deletion constructs of this fragment (Fig. 1), transformed flies, and assayed for reporter gene expression in early embryos (Fig. 2). The results show one

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construct that directs a posteriorly expanded reporter gene expression without altering the anterior border, a second construct that directs an anteriorly expanded expression without altering the posterior border, and a third construct that directs reporter gene expression throughout the embryo. This finding indicates that *kni* has the potential to be expressed throughout the wild-type embryo. To bring about the spatially restricted pattern, therefore, *kni* expression must be suppressed from the anterior and posterior sides, and separate cis-acting elements are needed to effect this repression.

We do not know what initially activates *kni* expression, and no plausible candidates exist among the genetically identified genes. However, in view of the observation that *kni* can be ubiquitously expressed, it is possible that no such external factor exists, and that *kni* becomes activated through an "internal autonomous" mechanism as discussed by Summerbell *et al.* (*11*). As for factors that are responsible for spatially restricting *kni* expression, genetic studies indicate that the gap genes *hb* and *tailless* (*tll*) may set the anterior and posterior borders, respectively (*10, 12*). The protein encoded by *hb* is found in the anterior half of the embryo, whereas *tll* is expressed in the terminal regions (*13*). In *hb* mutants, *kni* expression is expanded anteriorly, whereas in *tll* mutants it is expanded posteriorly (*10, 12*). This suggests that the *kni* upstream fragment whose deletion results in a posterior expansion of reporter gene expression in wild-type embryos (Fig. 1) might mediate repression by *tll*. By similar reasoning, the fragment whose deletion results in an anterior expansion of reporter gene expression in wild-type embryos (Fig. 1) might mediate repression by *hb*. In vitro footprinting studies (Fig. 3) lend further support to the view that these cis-acting elements may mediate some of *hb* and *tll* activities: *hb* protein binds to a fragment required for setting the anterior border, whereas *tll* protein binds to a fragment setting the posterior border. Further analysis (Fig. 4) suggests that some of the *hb* binding sites may function redundantly (*14*).

These results indicate that the mechanism by which *kni* is spatially regulated in the posterior region differs significantly from the one that regulates *hb* in the anterior. In the latter case the morphogen *bcd* forms a gradient within the anterior region of the embryo and, when present above a certain threshold concentration, directly activates *hb* expression (*6*). It has been postulated that other genes acting in the anterior region of the embryo are controlled by the *bcd* gradient in a manner analogous to *hb*, and several such genes have been genetically identified (*15*). The gap gene *Krüppel*, which is expressed in the central region of the embryo, also appears to be positively regulated by the local activity of the anterior morphogen system (*12, 16, 17*). By contrast, our results suggest that *kni* activation occurs throughout the embryo. The spatial borders are then actively set through repression by factors that are regionally localized. Once the initial activation and the spatial borders are established, additional factors such as *Kr*, a local transacting enhancer of *kni* gene expression, refines and modulates the expression pattern (*10*). Other genes acting in the posterior region (*18*), such as the gap gene *giant*, may be regulated in a manner analogous to *kni*.

The above results are consistent with the genetic data suggesting that the anterior and posterior maternal determinants function by fundamentally different mecha-

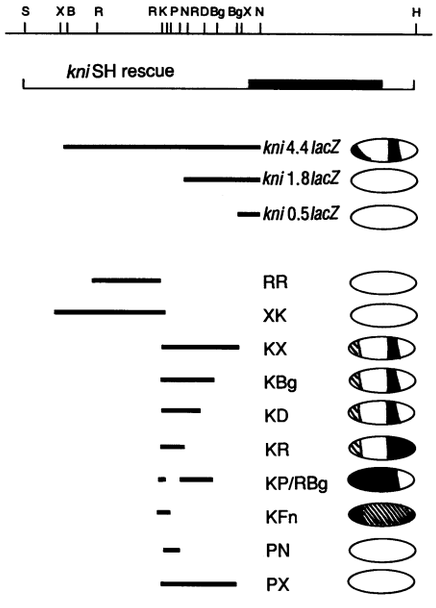

Fig. 1. Transformation constructs derived from the *kni* genomic region. Top line shows the positions of the various restriction sites utilized for making the constructs. The black bar in the 9-kb *kni*SH rescue fragment denotes the transcribed region. This construct, which contains the coding region and approximately 6 kb of flanking sequences, fully rescues the *kni* mutant phenotype (*22*). The thick lines on the left indicate the fragments used to make the *lacZ* fusion constructs (*23*). The first three constructs contain the endogenous *kni* promoter. All others are fused to the basal heat shock promoter (*23*). The schematized embryos on the right illustrate the β-gal expression patterns directed by the corresponding fusion constructs. Striped areas denote weaker staining. S, Sal I; X, Xba I; B, Bam HI; K, Kpn I; P, Pst I; N, Nru I; D, Dra I; Bg, Bgl II; H, Hind III; T, Taq I; C, Cla I; R, Eco RI; and Fn, Fnu 4HI.

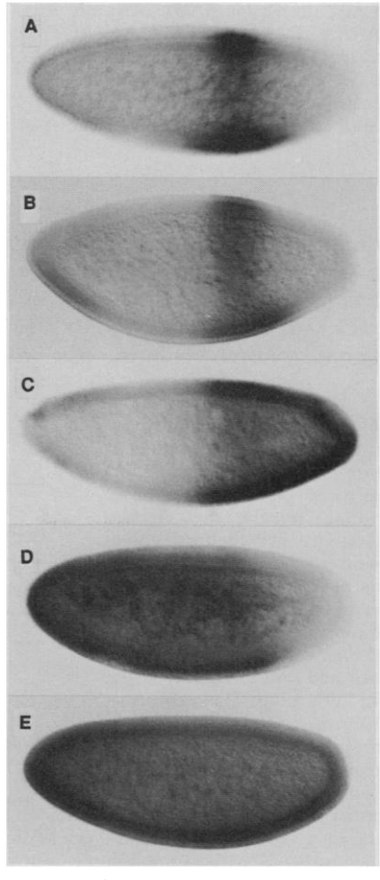

Fig. 2. Reporter gene expression directed by various *kni* upstream sequences in early embryos. Embryos are oriented with anterior to the left and dorsal up and were stained with anti-β-gal antibody as described (*24*). (**A**) KBg construct; the spatial pattern of the posterior expression resembles that of the *kni* transcripts. (**B**) KD construct; the spatial pattern is similar to the above KBg construct. (**C**) KR construct; the posterior domain is expanded to the posterior tip of the embryo. At late blastoderm the posterior domain retracts from the posterior tip of the embryo; this is also seen with endogenous *kni* expression in embryos derived from *torso* females (*25*). (**D**) KP/RBg construct; the posterior domain is expanded to the anterior region of the embryo. As *kni* is also expressed at the anterior tip (*9, 10*), we do not know to what extent the strong signal in the very anterior region is due to the increased expression of the anterior domain, the anteriorly expanded posterior domain, or both. However, the ectopic expression between approximately 50 to 75% egg length (posterior pole is 0%) is due to the anteriorly expanded posterior domain because that expression is still present in embryos derived from *bcd* mutant mothers (*25*). The endogenous *kni* expression (*12*) as well as the reporter gene expression of the *kni*4.4*lacZ* construct (*25*) in the anterior cap domain are absent in *bcd* embryos. (**E**) KFn construct; reporter gene is expressed throughout the embryo. As in the KP/RBg construct above, this construct also directs strong expression in the anterior *kni* domain. Constructs PN and PX, which both delete a common 58-bp Kpn/Pst fragment, did not direct any reporter gene expression.

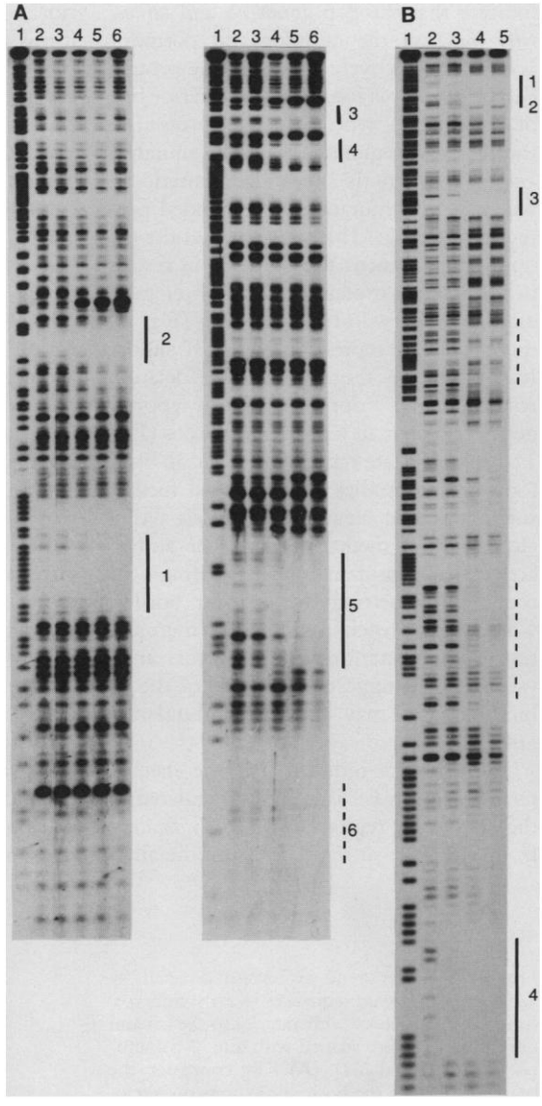

Fig. 3. (**A**) In vitro binding of *hb* protein to *kni* regulatory regions. Deoxyribonuclease I footprinting assay (*26*) was performed with a full-length *hb* protein on a 205-bp Pst I–Nru I (left panel) and 235-bp Nru I–Eco RI (right panel) subfragments that make up the 440-bp Pst I–Eco RI fragment required for setting the anterior border of *kni* expression (Fig. 1). Lanes 1, G + A marker; lanes 2, no added protein extract; lanes 3, 7.5 μl of control extract; and lanes 4 through 6, 0.25, 1.0, and 5.0 μl of *hb* extract, respectively. All protected areas (dotted line indicates weaker binding) contain previously established consensus sequence for *hb* binding (*27*): site 1, AATAAAAA; site 2, CAAAAAAC; site 3, CAAAAAAC; site 4, ATAAAAAA; site 5, GTAAAAAA; and site 6, GGAAAAAA. (**B**) In vitro binding of *tll* protein to *kni* upstream region. DNase I footprinting was performed with a truncated *tll* protein containing the finger domain on the 380-bp Eco RI–Dra I fragment required for setting the posterior border of *kni* expression (Fig. 1). Lane 1, G + A marker; lane 2, 15 μl of control extract; and lanes 3 through 5, 1, 7.5, and 15 μl of *tll* extract, respectively. With very high amounts of the *tll* extract additional areas become protected (lanes 4 and 5, dotted lines), representing weaker affinity *tll* binding sites. Footprinting was also performed on the other strand. The strongly protected areas (solid lines) contain the following sequences: site 1, GAAAGTCAA; site 2, GGAAGTCAA; site 3, AAAAGTCAA; and site 4, AAAAGTCAA. These sequences are in agreement with the consensus *tll* binding sequence derived from binding studies of *tll* protein to the Krüppel promoter (*28*).

nisms (7). This difference in organizing the two regions of the embryo most likely reflects the evolutionary history of *Drosophila*, a higher dipteran (*7, 19*). In more primitive insects, only the primordia for the anterior region are established all at once during blastoderm; the primordia for the abdominal segments, by contrast, form sequentially, presumably by cellular outgrowth from a posteriorly located growth zone (*20*). The different mechanisms underlying *hb* and *kni* regulation in *Drosophila* may reflect this evolutionary heritage. Elucidating how *kni* becomes activated should be essential for understanding how segments are generated within a growing field of primordial cells. In particular, a morphogenetic system based on propagation of positional information through diffusion, such as with *bcd* and *hb* (*4, 6*), would not be feasible in a cellular environment. However, if the functionally equivalent genes of *kni* in primitive insects become activated by an internal autonomous mechanism (*11*) as mentioned previously for *kni*, propagating this information could be accomplished essentially through the process of cell division itself (*21*).

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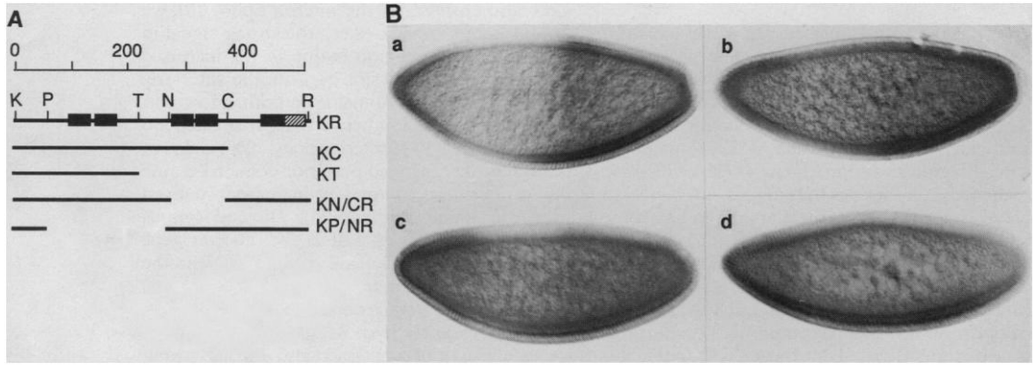

Fig. 4. Reporter gene expression of *kni* constructs with deletions of the *hb* binding sequences. (**A**) Locations of *hb* protein binding sites (rectangles; hatched figure denotes weaker binding site) within the *kni* upstream region and the various deletion constructs. (**B**) Expression patterns directed by the *kni* constructs. Embryos are oriented with anterior to the left and dorsal up. The a construct KC; there is no significant ectopic expression in the anterior region and KC resembles the pattern directed by the construct KR. The b construct KT; significant ectopic expression is detectable in the anterior region, but not as much as observed in construct KP/RBg. The c construct KN/CR; some ectopic expression is detectable in the anterior region, but the level appears weaker than in construct KT. The d construct KP/NR; there is an almost uniform level of expression in the embryo, except for the stronger level at the very anterior tip.

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14. The *hb* binding sites are clustered in tight pairs. Deletion of the different pairs results in varying degrees of ectopic expression in the anterior region, suggesting that the different clusters may function with varying degrees of efficiency in their ability to effect repression. The 5′ most cluster seems to be essential for repression because its deletion results in almost complete ectopic expression in the anterior region (construct KP/NR); however, this cluster is not sufficient to provide complete repression (construct KT). The middle cluster appears to increase the efficiency of repression, because the presence of both 5′ and middle clusters (construct KC) results in almost total repression of ectopic gene expression in the anterior region; consistent with this, deletion of the middle cluster (KN/CR) results in some ectopic anterior expression. These results also indicate that the 3′ most cluster functions least effectively because its deletion has almost no effect (construct KC), but, in conjunction with the 5′ cluster, can provide some repression function (construct KN/CR). Taken together, these results suggest that the different *hb* sequences are required to efficiently repress *kni* expression, perhaps by redundant use of regulatory elements [G. Schaffner, S. Schirm, B. Müller-Baden, F. Weber, W. Schaffner, *J. Mol. Biol.* **201**, 81 (1988)].
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21. This mechanism must of course be repressed in the nuclei or cells that will give rise to nonabdominal parts of the body. This could be accomplished during syncytial blastoderm by factors, perhaps functional homologs of *hb* and *tll*, in the anterior and terminal regions of the embryo. The major conceptual requirement would be that at the time of cellularization, at least one cell forms which is not repressed by these factors. This would then give rise to the future abdominal segments through cell division.
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29. We thank D. Tautz and C. Thummel for vectors, M. Rothe for communicating the *kni* rescue results before publication, and G. Brönner, G. Jürgens, M. Rothe, R. Sommer, and D. Tautz for comments and discussions. The *tll* expression vector was provided by E. Steingrimsson and J. Lengyel before publication. Supported by grants from the Deutsche Forschungs Gemeinschaft.

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The *fms*-Like Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor

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The *fms*-like tyrosine kinase (Flt) is a transmembrane receptor in the tyrosine kinase family. Expression of *flt* complementary DNA in COS cells conferred specific, high-affinity binding of vascular endothelial growth factor, also known as vascular permeability factor (VEGF-VPF), a factor that induces vascular permeability when injected in the guinea pig skin and stimulates endothelial cell proliferation. Expression of Flt in *Xenopus laevis* oocytes caused the oocytes to release calcium in response to VEGF-VPF. These findings show that *flt* encodes a receptor for VEGF-VPF.

VASCULAR PERMEABILITY FACTOR (VPF) was originally purified from guinea pig ascites and tumor cell culture medium as an agent that increases blood vessel permeability (*1, 2*). VPF also stimulates migration of monocytes across an endothelial cell monolayer (*3*). Independently, a mitogen specific for endothelial cells, termed vascular endothelial growth factor (VEGF), was purified from the conditioned medium of bovine pituitary folliculo stellate cells (*4, 5*). The amino acid sequences of VPF and VEGF are identical as predicted by their cDNA sequences, indicating that these different activities are embodied by the same molecule (*6–9*). VEGF-VPF is composed of two presumably identical chains held together by disulfide bonds. The amino acid sequence of VEGF-VPF is distantly related (18% overall identity) to the sequence of platelet-derived growth factor (PDGF), which is also a disulfide-linked dimer. The eight cysteines involved in intra- and interchain disulfide bridges in PDGF are identical in VEGF-VPF (*9, 11*). In contrast to other endothelial growth factors such as fibroblast growth factor (FGF) and platelet-derived endothelial cell growth factor (PD-ECGF), which do not have signal sequences for secretion by the endoplasmic reticulum pathway, VEGF-VPF has these sequences and is a secreted ligand (*12*).

We screened a cDNA library from human placenta with an oligonucleotide encoding a sequence common to many tyrosine kinases (*13*) and identified a cDNA clone, clone 9, which is almost identical to the published *fms*-like tyrosine kinase (*flt*) sequence (*14, 15*). Clone 9 and *flt* cDNAs encode proteins that are similar to the receptors in the PDGF receptor family, which include the CSF-1 receptor, the protein encoded by c-*kit*, and the PDGF α-type and β-type receptor (*16*). The similarities between the ligands VEGF-VPF and PDGF and between clone 9-*flt* and the PDGF receptor suggested that clone 9-*flt* might encode a receptor for VEGF-VPF.

The protein predicted by the *flt* cDNA would have seven immunoglobin-like domains in its extracellular region (compared to five immunoglobin-like domains in the other members of this receptor family), a single transmembrane spanning region, and a tyrosine kinase sequence that is interrupted by a kinase insert

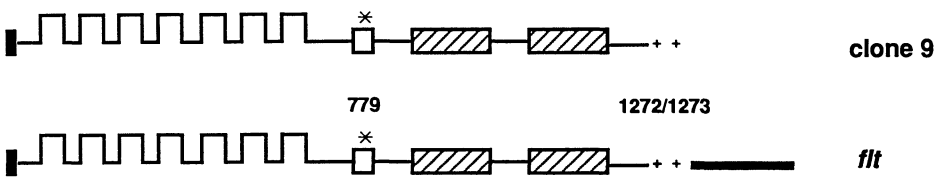

Fig. 1. Schematic representation of the structure of the proteins encoded by clone 9 and *flt*. The following structural features are identified: (■) hydrophobic leader sequence, immunoglobin-like sequence, (□) transmembrane membrane domain, (▨) kinase domains. The asterisk indicates the position in the transmembrane domain where the published *flt* sequence has Leu^{779} (*15*) and where our isolates of clone 9 and *flt* have Phe^{779}. The position in the COOH-terminal region at which clone 9 has Glu^{1272}-Val^{1273} and Flt has Asp^{1272}-Leu^{1273} is indicated (++). The thick line depicts the 65 additional amino acids at the COOH-terminus of Flt that are not present in clone 9.

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