34. A. I. Magee *et al.*, *Biochim. Biophys. Acta* **798**, 156 (1984).
35. M. F. G. Schmidt, M. Bracha, M. J. Schlesinger, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1687 (1979).
36. E. Kraig, T. Dailey, D. Kolodrubetz, *Infect. Immun.* **58**, 920 (1990); J. Frey, R. Meier, D. Gygi, J. Nicolet, *ibid.* **59**, 3026 (1991).
37. We thank E. Koronakis for technical assistance. Funded by a Medical Research Council Programme grant to C.H. and V.K.

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Coordinate Initiation of *Drosophila* Development by Regulated Polyadenylation of Maternal Messenger RNAs

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Pattern formation in *Drosophila* depends initially on the translational activation of maternal messenger RNAs (mRNAs) whose protein products determine cell fate. Three mRNAs that dictate anterior, dorsoventral, and terminal specification—bicoid, Toll, and torso, respectively—showed increases in polyadenylate [poly(A)] tail length concomitant with translation. In contrast, posteriorly localized nanos mRNA, although also translationally activated, was not regulated by poly(A) status. These results implicate at least two mechanisms of mRNA activation in flies. Studies with bicoid mRNA showed that cytoplasmic polyadenylation is necessary for translation, establishing this pathway as essential for embryogenesis. Combined, these experiments identify a regulatory pathway that can coordinate initiation of maternal pattern formation systems in *Drosophila*.

In *Drosophila*, four maternal systems define initial embryonic asymmetry: anterior, posterior, dorsoventral, and terminal (*1*). Formation of the anteroposterior axis is guided by the localization of two crucial mRNAs, bicoid in the anterior end (*2, 3*) and nanos in the posterior end (*4*). The dorsoventral and terminal systems each contain a uniformly distributed mRNA—Toll (*5*) and torso (*6*), respectively—encoding a transmembrane receptor (*5, 7*) that responds to localized ligand to create asymmetry (*8*).

These four mRNAs—bicoid, nanos, Toll, and torso—that determine regional specification all exhibit translational activation during early embryogenesis (*3, 6, 9, 10*). Translational activation of maternal mRNAs can be controlled by various processes (*11*), but a predominant mechanism is cytoplasmic elongation of the poly(A) tail (*12, 13*). Cytoplasmic polyadenylation occurs in many species, suggesting it is an ancient and conserved form of mRNA activation during oocyte maturation and embryogenesis. However, it has not been possible to demonstrate an essential role for this regulation in development. To address this question, we have exploited the extensive knowledge of *Drosophila* maternal mRNAs as well as the ability to assess mRNA translation in vivo by injection of mutant embryos.

We used a polymerase chain reaction (PCR)–based assay [poly(A) test (PAT)] to determine whether *Drosophila* mRNAs exhibit changes in poly(A) status upon translational activation (*14*). The size of the PCR products in this assay reflects the poly(A) tail length of the RNA. The assay was applied to four maternal mRNAs—oskar, exuperantia, α4-tubulin, and bicoid—the first three of which are translated during oogenesis (*15*) and were not expected to undergo an elongation of their poly(A) tails during embryogenesis. Bicoid mRNA was a candidate for cytoplasmic polyadenylation because it is translationally regulated (*3*), and in BicD mutant embryos suppression of bicoid mRNA translation is correlated with a short poly(A) tail (*16*).

RNA was isolated from ovaries and embryos at various developmental stages and subjected to PAT analysis (Fig. 1). In oocytes, bicoid mRNA had a poly(A) tail of ~70 nucleotides (nt). Because bicoid mRNA is dormant at this stage, this poly(A) tail length is not sufficient for translation. The length of the poly(A) tail increased within 1 hour after embryo deposition, peaked at ~140 nt in 1 to 1.5-hour embryos, and then progressively shortened (Fig. 1A). The time course of polyadenylation is compatible with a role in translational activation of bicoid mRNA; bicoid protein is first observed in 1- to 2-hour embryos, reaches a maximum at 2 to 4 hours, and then disappears (*3*). Although oskar mRNA contained a poly(A) tail of ~100 nt in oocytes, it was not further elongated during embryogenesis (Fig. 1B); similarly, exuperantia and α4-tubulin mRNAs did not exhibit cytoplasmic polyadenylation. Thus, the increase in poly(A) tail length does not occur in these constitutively translated mRNAs. These data reveal a correlation between cytoplasmic polyadenylation and translational activation during *Drosophila* embryogenesis.

To determine whether polyadenylation controls translational activation of bicoid mRNA, we analyzed the rescuing ability and the poly(A) status of injected bicoid transcripts. Embryos derived from females homozygous for the bcd^{E1} mutation do not produce functional bicoid protein (*17*) or form anterior structures (head and thorax) (*2*). Therefore, the requirements for bicoid mRNA translation can be examined by injecting synthetic transcripts into bcd^{E1} embryos and determining rescue of anterior structures (*18*). Injection of the wild-type bicoid (bcd^{wt}) transcript (Fig. 2) into the anterior region of bcd^{E1} embryos fully rescued the mutant embryos (Fig. 3, A and G), yielding, in some instances, fertile adults. This observation indicated that bcd^{wt} RNA is translated effectively in vivo (*19*).

Determination of the requirement for polyadenylation in translation necessitated the preparation of a nonrescuing bicoid transcript. Because the regulatory sequences for polyadenylation often reside in the 3′ untranslated region (UTR), it was probable that a transcript lacking this region would be inactive. Therefore, an mRNA truncated by 537 nt in the 3′ UTR [at the Sma I site of the complementary DNA (cDNA)] (bcd^{Sma}) was generated (Fig. 2). This shortened bicoid transcript did not rescue mutant embryos (Fig. 3B); even at a 10-fold higher concentration (2 μg/μl), bcd^{Sma} RNA showed essentially no rescue (*20*). The effect of poly(A) tail length on translation was then investigated. bcd^{Sma} RNAs with different poly(A) tail lengths were generated by in vitro polyadenylation (Fig. 2): $bcd^{Sma+A_{50}}$ RNA contained 40 to 60 A residues, which approximates the poly(A) tail on the endogenous, translationally silent, oocyte mRNA; $bcd^{Sma+A_{175}}$ RNA contained 150 to 200 A residues, similar to the poly(A) tail on the endogenous, active, embryonic mRNA. The $bcd^{Sma+A_{50}}$ RNA had no effect on the bcd^{E1} phenotype when injected into mutant embryos (Fig. 3C). In contrast, $bcd^{Sma+A_{175}}$ RNA effectively rescued, with all three thoracic segments often present as well as gnathal and head structures (Fig. 3, D and H).

Although $bcd^{Sma+A_{175}}$ RNA partially

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rescued the bcd^{E1} phenotype, it was less effective than bcd^{wt} RNA. One possible explanation for this result is that the truncated RNA lacks sequences required for optimal translation, even in the presence of a long poly(A) tail. For example, the translation of bicoid mRNA may be enhanced via interaction with staufen protein (*21*); this interaction occurs via the 3′ UTR of bicoid mRNA, which is missing in $bcd^{Sma+A_{175}}$ RNA.

If cytoplasmic polyadenylation is required for translation of bicoid mRNA, then the rescuing bcd^{wt} mRNA should be elongated after injection. To test this prediction, we injected ^{32}P-labeled bcd^{wt} mRNA into the anterior region of bcd^{E1} embryos; 1 hour later, the mRNA was retrieved (*22*) and examined (Fig. 4). The transcript was polyadenylated after injection with ~150 A residues. In contrast, bcd^{Sma}, $bcd^{Sma+A_{50}}$, and $bcd^{Sma+A_{175}}$ RNAs, which do not contain the 3′ UTR sequences, were not significantly elongated (<50 nt). These results indicate the crucial role of poly(A) status in translation, and that sequences in the 3′ UTR regulate efficient cytoplasmic polyadenylation.

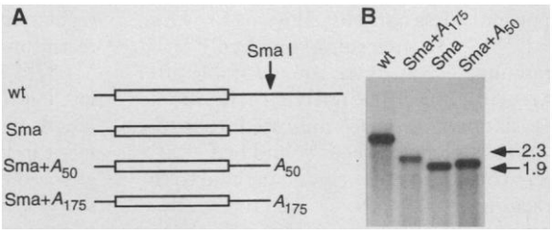

Fig. 2. (A) The bicoid transcripts used to determine the role of polyadenylation in translational activation: wt, full-length bicoid mRNA showing the cDNA Sma I site used to generate truncated transcripts; Sma, bicoid mRNA lacking the 3′-terminal 537 nt; Sma+A_{50} and Sma+A_{175}, Sma transcripts polyadenylated in vitro to contain ~50 or ~175 A residues, respectively. Open box, coding region. Capped transcripts labeled with [α-^{32}P]UTP (uridine triphosphate) were synthesized with a T3 Message Machine kit (Ambion) (specific activity, 5×10^3 cpm/μg), purified, and resuspended in filtered water at 0.2 μg/μl. In vitro polyadenylation was performed with 5 μg of heat-denatured bcd^{Sma} RNA and 300 μM adenosine triphosphate (ATP) (*13*) for either 2 min ($bcd^{Sma+A_{50}}$) or 20 min ($bcd^{Sma+A_{175}}$) at 37°C. Unincorporated ATP was removed by two rounds of ethanol precipitation. **(B)** Size and integrity of transcripts. RNAs were visualized by autoradiography after electrophoresis in formaldehyde-agarose gels and transfer to Duralon membranes (Stratagene). The extent of polyadenylation was estimated from the mobility of DNA markers (2.3 and 1.9 kb, arrows) parent RNAs.

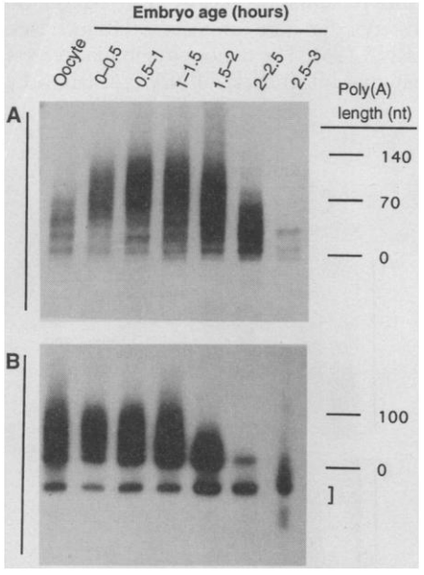

Fig. 1. PAT analysis of bicoid (**A**) and oskar (**B**) mRNA during *Drosophila* embryogenesis. Total RNA was isolated (*30*) from *Drosophila* ovaries and embryos. Each RNA sample was treated with deoxyribonuclease (*25*), purified, and subjected to PAT cDNA synthesis (*14*). All transcript-specific amplifications were performed on the same cDNA preparation (mRNA primers: bicoid, 5′-CATTTGCGCATTCTTTGACC; oskar, 5′-AAGCGCTTGTTTGTAGCACA). Because the amount of RNA was not determined for each time point, quantitative estimates cannot be made. Poly(A) tail lengths are reported as maximally elongated species; similar conclusions were drawn if the poly(A) tail lengths were estimated as the average elongation. The size of bicoid amplified PAT products without poly(A) is 286 base pairs (256 bicoid plus 30 anchor). The size of oskar amplified PAT products is 253 base pairs (223 oskar plus 30 anchor). An apparent deadenylation is observed between 1.5 and 2.5 hours with oskar mRNA; the lower band (bracket) at 220 base pairs is a secondary oskar-specific amplification product. Tail lengths were estimated from adjacent molecular size markers.

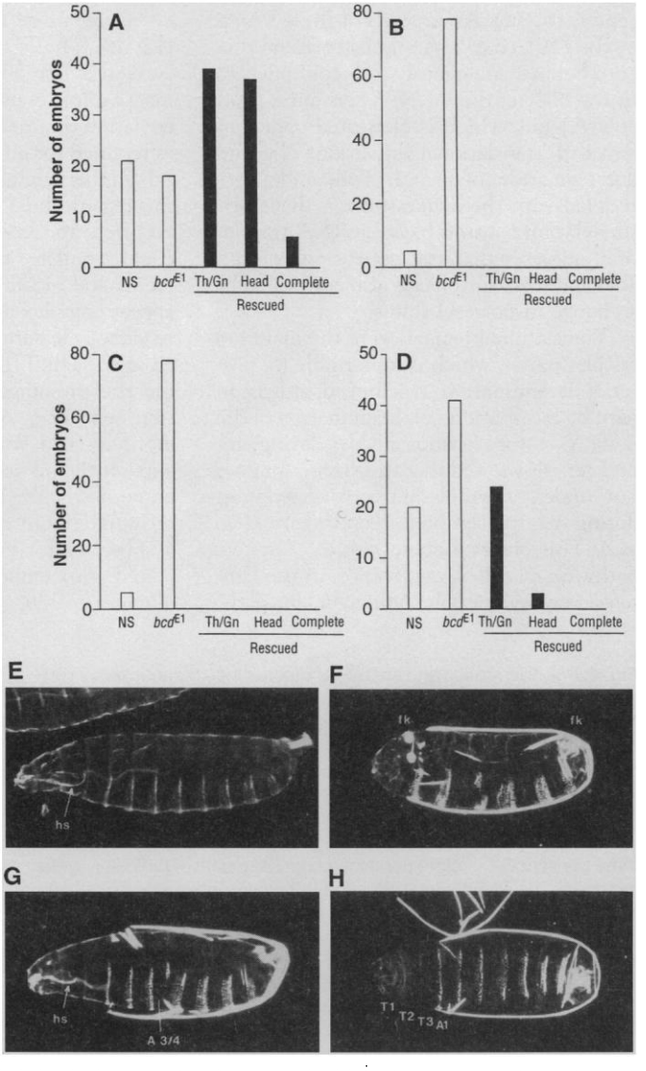

Fig. 3. (**A** to **D**) Degree of rescue of bcd^{E1} embryos injected with wild-type (A), Sma (B), Sma+A_{50} (C), or Sma+A_{175} (D) bicoid transcripts. Filled bars denote rescued embryos. RNAs (0.2 μg/μl in H_2O) (Fig. 2) were injected anteriorly (*2*) at room temperature into 0- to 0.5-hour bcd^{E1} embryos, which were then incubated for 22 to 26 hours at room temperature. Embryos were scored (*2, 3, 18*) as follows: NS, cuticle phenotype not scorable; bcd^{E1}, no rescue, filzkörper duplicated at anterior end, equivalent to uninjected bcd^{E1} embryos; Th/Gn, thoracic/gnathal structures; Head, appearance of antennal sense organs with or without partial head skeleton; and Complete, hatched larva or normal cuticle with complete head skeleton. (**E** to **H**) Cuticle preparations of rescued embryos. Cuticles in Hoyers medium: lactic acid (1:1) were visualized with dark-field optics (left, anterior). (E) Wild-type embryo; all anterior structures present. (F) Uninjected bcd^{E1} embryo; partial duplication of the posterior filzkörper (fk) at the anterior end. (G) Mutant embryo injected anteriorly with bcd^{wt} transcript; rescued embryo with almost complete head skeleton (hs) and fused abdominal segments A3 and A4. (H) Mutant embryo injected anteriorly with $bcd^{Sma+A_{175}}$; three thoracic segments (T1 to T3) rescued with no gnathal or head structures (*31*).

The differences in rescuing ability of the various transcripts cannot be explained by differences in half-life or inherent in vitro translatability. All RNAs showed similar half-lives after injection, with the exception of bcd^{Sma}, which appeared less stable (Fig. 4). Thus, $bcd^{Sma+A_{50}}$ (nonrescuing) and $bcd^{Sma+A_{175}}$ (rescuing) RNAs were equally stable after injection. Additionally, reticulocyte translations in vitro indicated that the nonrescuing RNAs bcd^{Sma} and $bcd^{Sma+A_{50}}$ were translated at levels comparable to the rescuing bcd^{wt} RNA. Taken together, these results demonstrate that translational activation of bicoid mRNA during *Drosophila* embryogenesis requires cytoplasmic polyadenylation.

Pivotal mRNAs in the other maternal pattern formation pathways were next examined for cytoplasmic polyadenylation during development. Both torso (terminal) and Toll (dorsoventral) mRNAs are translationally activated during early embryogenesis (*6*, *10*). An analysis of these RNAs by the PAT (Fig. 5, A and B) revealed that both behave analogously to bicoid mRNA: In the oocyte, the mRNAs contain a short poly(A) tail, which is elongated concomitant with translational activation. The similar regulation of bicoid, Toll, and torso, coupled with the demonstration that polyadenylation controls bicoid mRNA translation, suggests that the activation of both Toll and torso mRNAs is also controlled by a change in poly(A) status.

Translational regulation of the maternal mRNA nanos, which is responsible for posterior determination, is achieved, at least in part, by localization (9). Examination of the poly(A) status of nanos mRNA during embryogenesis showed that this transcript does not undergo cytoplasmic polyadenylation during its translational recruitment (Fig. 5C). This observation reveals at least two pathways for mRNA activation in the *Drosophila* embryo: a poly(A)-dependent pathway represented by bicoid, Toll, and torso, and a second system for nanos.

Our results show that regulation of cytoplasmic polyadenylation is a fundamental mechanism of translational control in *Drosophila*. Previous evidence of this form of regulation in flies is that the translational recruitment of two mRNAs (Mst87D and Mst87F) in postmeiotic spermatids is correlated with an increase in poly(A) tail length (*23*). In these mRNAs, a 12-nt element in the 5′ UTR both regulates polyadenylation and represses translation in primary spermatocytes. This element is not present in bicoid mRNA, and replacement of the 5′ UTR of bicoid mRNA with heterologous sequences does not relieve the translational repression in oocytes (*18*).

Studies in *Xenopus* and mouse demonstrate that cytoplasmic polyadenylation requires two sequences: the canonical nuclear polyadenylation-cleavage signal, AAUAAA, and an AU-rich element [cytoplasmic polyadenylation element (CPE), or adenylation control element (ACE)] (*12*, *13*, *24*, *25*). The bcd^{Sma} RNA, which lacks the last 537 nt of the 3′ UTR, is inactive for rescue and shows little polyadenylation on injection. Therefore, sequences required for efficient polyadenylation reside in this deleted region of the 3′ UTR. In this regard, the 3′ UTR of bicoid mRNA is AU-rich and contains many regions that could regulate translation. The requirements for bicoid mRNA polyadenylation appear complex and to encompass multiple sequence elements. A transcript (terminated at the Hind III site of the cDNA) lacking the presumptive nuclear polyadenylation sequence, AAUAUA, and an ACE motif rescued bcd^{E1} mutant embryos with apparently no loss of efficiency as compared to bcd^{wt} RNA. Additionally, the terminal 729 nt of bicoid mRNA were not a substrate for polyadenylation when injected into embryos (*26*). Therefore, sequences both upstream and downstream of the Sma I site are required for cytoplasmic polyadenylation.

Sequence comparison of the 3′ UTRs in the mRNAs regulated by polyadenylation in *Drosophila* does not reveal significant homology. In addition, a chimeric mRNA in which the entire 3′ UTR of bicoid mRNA was replaced with the corresponding region from the α1-tubulin mRNA exhibits normal translational regulation (*27*). Therefore, an exact definition of the sequences that participate in bicoid, Toll, and torso translational regulation awaits further analysis.

Translational activation of nanos mRNA does not involve cytoplasmic polyadenylation. Expression of nanos mRNA depends on proper posterior localization, with translational suppression occurring when the transcript is mislocalized (9). Therefore, posterior positioning may be sufficient for translation of nanos mRNA. In embryogenesis, nanos protein is necessary only to repress the posterior function of maternal hunchback mRNA (*28*). The nanos protein may repress maternal hunchback mRNA translation by

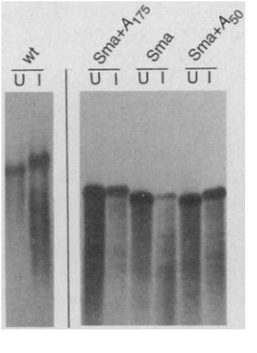

Fig. 4. Polyadenylation and stability of injected bicoid transcripts. High–specific activity transcripts (2×10^7 to 3×10^7 cpm/μg) were generated (1 hour at 37°C) with a T3 MegaScript kit (Ambion) and 0.5 mM adenosine triphosphate, 0.5 mM cytidine triphosphate, 0.25 mM uridine 5′phosphate (UTP), 0.25 mM guanosine triphosphate, 1.0 mM $m7G(5')_{ppp}(5')G$ (CAP analog) and [α-^{32}P] UTP. The RNAs were polyadenylated, purified, and injected as in Figs. 2 and 3. The bcd^{Sma} RNA was used to generate both $bcd^{Sma+A_{50}}$ and $bcd^{Sma+A_{175}}$ RNAs; these three transcripts therefore contained equal amounts of radioactivity per molecule. The RNAs were reisolated (*22*) after 1 hour (lanes I), and 11 embryo-equivalents were subjected to electrophoresis with uninjected RNAs as size markers (lanes U) to determine in vivo polyadenylation of injected transcripts. Therefore, for the three Sma transcripts, the amounts of RNA remaining after 1 hour (lanes I) are directly comparable. Quantitation (means ± SE, $n = 3$) of the relative amount of full-length RNA for the three Sma transcripts after retrieval was as follows (normalized to $bcd^{Sma+A_{50}}$ RNA) $bcd^{Sma+A_{50}}$, 1.0; bcd^{Sma}, 0.43 ± 0.04; and $bcd^{Sma+A_{175}}$, 1.18 ± 0.39. The bcd^{Sma} RNA showed no rescue at a concentration 10 times that of rescuing transcripts, and therefore would have to be less than one-tenth as stable to explain the results on the basis of stability alone.

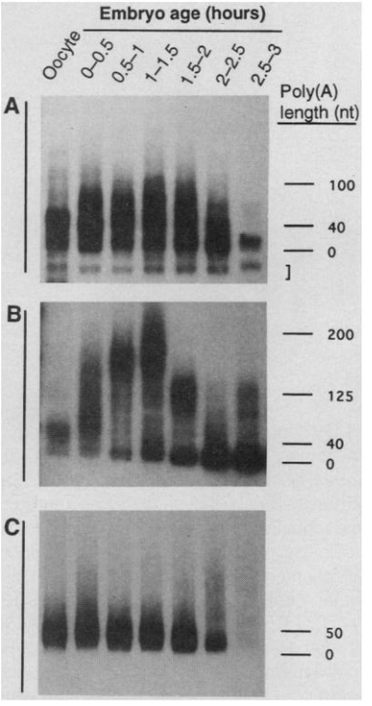

Fig. 5. PAT analysis of torso (**A**), Toll (**B**), and nanos (**C**) RNAs. The sizes of the amplified PAT products [minus poly(A)], length of poly(A) tail in oocytes, and length of poly(A) tail in embryos are 305, 40, and 100 nt (torso); 335, 40, and 200 nt (Toll); and 350, 50, and 50 nt (nanos), respectively. The lower band (bracket) in (A) is a secondary torso-specific PCR amplification product. PCR primers: torso, 5′-CCAGAAAGGCTGAAACAACTGCAAG; Toll, 5′-GTATCAACTGTAATCTCACGCCCA; and nanos, 5′-GTCGTCGGCTACGCATTCATTGT.

alteration of its poly(A) status (*16*), and we have shown that hunchback mRNA is polyadenylated during development (*29*). Because hunchback mRNA is translationally activated at egg deposition and nanos protein should appear before hunchback protein, a poly(A)-independent mechanism for nanos mRNA might ensure earlier synthesis of this protein.

Polyadenylation of maternal mRNAs is a conserved mechanism of translational activation during oogenesis and early embryogenesis. Therefore, mutations in the pathway might behave as female-sterile mutations, given that maternal mRNAs that require cytoplasmic polyadenylation would not be translationally activated. The isolation and characterization of such *Drosophila* mutants should allow definition of gene products and biochemical pathways that control the initiation of development.

REFERENCES AND NOTES

1. D. St Johnston and C. Nüsslein-Volhard, *Cell* **68**, 201 (1992).
2. H. G. Frohnhöfer and C. Nüsslein-Volhard, *Nature* **324**, 120 (1986).
3. W. Driever and C. Nüsslein-Volhard, *Cell* **54**, 83 (1988).
4. C. Wang and R. Lehmann, *ibid.* **66**, 637 (1991); C. Wang, L. K. Dickinson, R. Lehmann, *Dev. Dynam.* **199**, 103 (1994).
5. C. Hashimoto, K. Hudson, K. V. Anderson, *Cell* **52**, 269 (1988).
6. J. Casanova and G. Struhl, *Genes Dev.* **3**, 2025 (1989).
7. F. Sprenger, L. M. Stevens, C. Nüsslein-Volhard, *Nature* **338**, 478 (1989).
8. J.-R. Martin, A. Raibaud, R. Ollo, *ibid.* **367**, 741 (1994); D. Morisato and K. V. Anderson, *Cell* **76**, 677 (1994); D. Stein, S. Roth, E. Vogelsang, C. Nüsslein-Volhard, *ibid.* **65**, 725 (1991); L. Stevens, H. Frohnhöfer, M. Klinger, C. Nüsslein-Volhard, *Nature* **346**, 660 (1990).
9. E. R. Gavis and R. Lehmann, *Nature* **369**, 315 (1994).
10. N. J. Gay and F. J. Keith, *Biochim. Biophys. Acta* **1132**, 290 (1992).
11. N. Standart, *Sem. Dev. Biol.* **3**, 367 (1992).
12. M. Wickens, *ibid.*, p. 399; J. D. Richter, *BioEssays* **13**, 179 (1991); R. R. Bachvarova, *Cell* **69**, 895 (1992).
13. J.-D. Vassalli *et al.*, *Genes Dev.* **3**, 2163 (1989).
14. F. J. Sallés and S. Strickland, in preparation; modification of assay described in F. J. Sallés, A. L. Darrow, M. L. O'Connell, S. Strickland, *Genes Dev.* **6**, 1202 (1992).
15. A. Ephrussi, personal communication; P. M. Macdonald, S. K.-S. Luk, M. Kilpatrick, *Genes Dev.* **5**, 2455 (1991); W. E. Theurkauf, H. Baum, J. Bo, P. C. Wensink, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8477 (1986).
16. R. P. Wharton and G. Struhl, *Cell* **67**, 237 (1991).
17. T. Berleth *et al.*, *EMBO J.* **7**, 1749 (1988).
18. W. Driever, J. Ma, C. Nüsslein-Volhard, M. Ptashne, *Nature* **342**, 149 (1989); W. Driever, V. Siegel, C. Nüsslein-Volhard, *Development* **109**, 811 (1990).
19. A putative "wild-type" bicoid transcript could not rescue when injected into *bcd*^{E1} embryos (*18*); rescue was observed only when the bicoid mRNA 5′ leader sequence was replaced by the *Xenopus* globin 5′ UTR. However, we found that the "wild-type" transcript used in the previous study contained, at its 5′ end, antisense sequences directed against the bicoid coding region. Therefore, a full-length bicoid cDNA clone was generated by replacing these 5′ antisense sequences with the 5′ wild-type sequences (*17*): A *Xenopus* globin 5′ UTR–bicoid cDNA construct (*18*) was cloned into pKS+ (pKS-bcdTN3). The bicoid 5′ UTR was amplified and used to replace the *Xenopus* globin leader (pKS-bcdTN3) to generate pBCD^{wt}. A T3 RNA polymerase transcript generated from Not I–linearized pBCD^{wt} contains 35 nt of polylinker, bicoid mRNA (nt 1–2496), a 14-nt poly(A) tail, and Not I–But II sequences.
20. No rescue, 67 embryos; partial rescue, 1 embryo.
21. D. Ferrandon, L. Elphick, C. Nüsslein-Volhard, D. St Johnston, *Cell*, in press.
22. E. Gottlieb, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7164 (1992).
23. R. Kuhn *et al.*, *Mech. Dev.* **35**, 143 (1991); M. Schäfer, R. Kuhn, F. Bosse, U. Schäfer, *EMBO J.* **9**, 4519 (1990).
24. C. A. Fox, M. D. Sheets, M. P. Wickens, *Genes Dev.* **3**, 2151 (1989); L. L. McGrew and J. D. Richter, *EMBO J.* **9**, 3743 (1990); L. L. McGrew, E. Dworkin-Rastl, M. B. Dworkin, J. D. Richter, *Genes Dev.* **3**, 803 (1989); J. Paris and J. D. Richter, *Mol. Cell. Biol.* **10**, 5634 (1990).
25. J. Huarte *et al.*, *Cell* **69**, 1021 (1992).
26. A. C. Verrotti, C. Wreden, S. Strickland, unpublished observations.
27. R. P. Wharton, *Sem. Dev. Biol.* **3**, 391 (1992).
28. M. Hülskamp, C. Schröder, C. Pfeifle, H. Jäkle, D. Tautz, *Nature* **338**, 629 (1989); V. Irish, R. Lehmann, M. Akam, *ibid.*, p. 646; G. Struhl, *ibid.*, p. 741.
29. F. J. Sallés, M. E. Lieberfarb, C. Wreden, J. P. Gergen, S. Strickland, unpublished observations.
30. J. Huarte, D. Belin, A. Vassalli, S. Strickland, J.-D. Vassalli, *Genes Dev.* **1**, 1201 (1987).
31. This extent of rescue represents the average observed; occasionally, gnathal or head structures were present.

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Trans-Acting Dosage Effects on the Expression of Model Gene Systems in Maize Aneuploids

Mei Guo and James A. Birchler*

The reduction in vigor of aneuploids was classically thought to be due to the imbalance of gene products expressed from the varied chromosome relative to those from the remainder of the genome. In this study, the dosage of chromosomal segments was varied, but the transcript level of most genes encoded therein showed compensation for the number of copies of the gene. Genes whose dosage was not altered were affected by aneuploidy of unlinked chromosomal segments. The phenotypic effects of aneuploidy and of a substantial fraction of quantitative variation are hypothesized to be the consequence of an altered dosage-sensitive regulatory system.

The phenotypic consequences of addition or subtraction of a chromosome relative to the normal genomic complement have been documented in a variety of eukaryotic organisms (*1–3*). The detrimental effects of aneuploidy were considered to be the result of the imbalance of the gene products encoded on the varied chromosome relative to those from the remainder of the genome (*1–3*). This concept was based on the assumption that the amount of gene products increases proportionally as the structural gene dose increases, a concept known as gene dosage effects. However, studies on *Alcohol dehydrogenase 1* (*Adh1*) and *Globulin 1* (*Glb1*) in maize (*Zea mays*) revealed that the expression levels of the ADH1 enzyme and GLB1 protein remained constant for various doses of the long arm of chromosome 1 (1L), where both structural genes reside (*4*, *5*). This lack of a structural gene dosage effect is referred to as dosage compensation. However, when the copy number of the respective structural genes was varied from one to four in a ploidy series, directly proportional expression amounts of *Adh1* and *Glb1* were observed relative to the gene copy number. In addition, most other aneuploids analyzed exhibited trans-acting dosage effects on the expression of genes encoded on unvaried chromosomes (*5*). We used a genetically marked dosage series of 14 chromosomal regions and several specific genes in *Z. mays* to test the effects of segmental ploidy on gene expression. Transcript amounts of the following genes were analyzed: *Adh1* (*6*, *7*), *Alcohol dehydrogenase 2* (*Adh2*) (*7*, *8*); *Glb1* (*9*), *Shrunken 1* (*Sh1*) (*10*), *Sucrose synthase 1* (*Sus1*) (*11*), and *Zein* (22 kD) (*12*).

The genetic system we used to generate the dosage series involved translocations between the supernumerary B chromosome and the normal A chromosomes marked with dominant anthocyanin pigment genes or their transposable element derivatives (*13*). The B chromosome frequently nondisjoins at the second pollen mitosis (*14*), which gives rise to the two sperm in a pollen grain, thus generating zero, one, or two copies of the B chromo-

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