that this activity can be antagonized non-cell autonomously by *sog*.

These experiments demonstrate that dorsal–ventral patterning in both *Drosophila* and *Xenopus* is dependent upon a system involving two extracellular proteins, Dpp/Bmp-4 and Sog/Chordin. Despite fundamental morphological differences between early embryos of the two species, by the late blastula/early gastrula stage the *sog/chordin* gene is expressed in each embryo on the side from which the CNS arises, while the *dpp/Bmp-4* gene is expressed in cells on the opposite side of the embryo. Moreover, the activity of each gene promotes development of the tissue type in which it is expressed. These results support the view that there was a reversal in the dorsal–ventral axis after the divergence of the common ancestor of insects and vertebrates[14–17]. □

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Activation of posterior gap gene expression in the *Drosophila* blastoderm

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THE process of body prepatterning during *Drosophila* blastoderm formation relies on the localized activities of zygotic segmentation genes, which are controlled by asymmetrically distributed maternal determinants[1,2]. The anterior determinant bicoid, a homeodomain transcription factor[3,4], forms an anterior-to-posterior concentration gradient[1–4]. It interacts with the maternal transcription factor hunchback[5] to activate the anterior zygotic patterning genes, including the central gap gene *Krüppel* (*Kr*)[6]. In contrast, the posterior maternal system[1,2] does not provide such a decisive transcription factor, but rather prevents the repressor hunchback from acting in the posterior half so that the gap genes *giant* (*gt*) and *knirps* (*kni*) are activated by an as yet unknown transcription factor[2,7]. Here we show that caudal, a conserved homeodomain protein that forms a posterior-to-anterior concentration gradient[8,9], and the anterior determinant bicoid cooperate to form a partly redundant activator system in the posterior region of the embryo.

In the search for factor(s) that activate posterior *kni* gene expression, we examined deletion constructs of the 2-kilobase (kb) *kni cis*-acting region[7] for their ability to conduct reporter gene expression in transgenic embryos. Figure 1 shows that gene activation in the *kni*-domain depends on two separate activator modules of 64 bp and 223 bp that respond to the homeodomain transcription-factor gradients provided by the anterior morphogen bicoid[1,2] and the posterior morphogen caudal (Fig. 1*a*, *b*), respectively. Reporter genes containing the kni64 element are expressed in *caudal* mutants but not in embryos lacking *bicoid* activity, whereas expression mediated by the kni223 element is affected in *caudal* embryos but not in embryos lacking bicoid (Fig. 1*c*, *d*). This suggests that caudal regulates transcription of *kni* through the kni223 element and bicoid mediates transcription via the kni64 element (Fig. 1*e*).

The kni64 and kni223 elements are not only necessary (Fig. 1*f*) but also sufficient to mediate gene activation in response to bicoid and caudal, respectively (Fig. 2*a*, *b*). However, the combined kni223/kni64 element conducts gene activation in the posterior but not in the anterior half (Fig. 2*c*). This indicates that the kni64 element is not able to mediate bicoid-dependent activation in the anterior region of the embryo when combined with the kni223 element. In fact, the kni223 element contains two binding sites for hunchback (Fig. 1*b*). As hunchback is a strong *kni* repressor[2,7], we deleted these binding sites. The resulting Δhb-kni223/kni64 element causes gene activation throughout the embryo (Fig. 2*d*). This finding suggests that hunchback represses directly through these binding sites and thereby restricts ubiquitous gene activation in response to the combined activities of bicoid and caudal.

The bicoid-responsive kni64 element contains six tightly clustered bicoid binding sites (Fig. 3*a–c*). The expression patterns of reporter genes that contain site-specific deletions and/or combinations of different binding sites for bicoid (Fig. 3*f–k*) indicate that four sites are sufficient to provide posterior gene expression, and that the ability to activate *kni*-like gene expression is not dependent on specific binding sites for bicoid, their orientation or their affinity (results not shown). Thus their specific arrangement within the element may allow them to sense the low bicoid concentrations in the posterior half of the embryo and to mediate gene activation by a currently unknown mechanism. In contrast, the caudal-responsive kni223 element contains six variably spaced binding sites for caudal, of different affinities (Fig. 3*d*, *e*). Two of the high-affinity sites are sufficient to mediate caudal-dependent gene activation (Fig. 3*l*). As caudal does not bind in a cooperative fashion (results not shown), we assume that the kni223 element mediates gene activation by the high-affinity binding sites, which respond to a critical threshold value of combined maternal and zygotic activities of caudal[2,7].

The phenotypes of embryos lacking caudal and bicoid activities indicate that neither is sufficient to establish a normal ab-

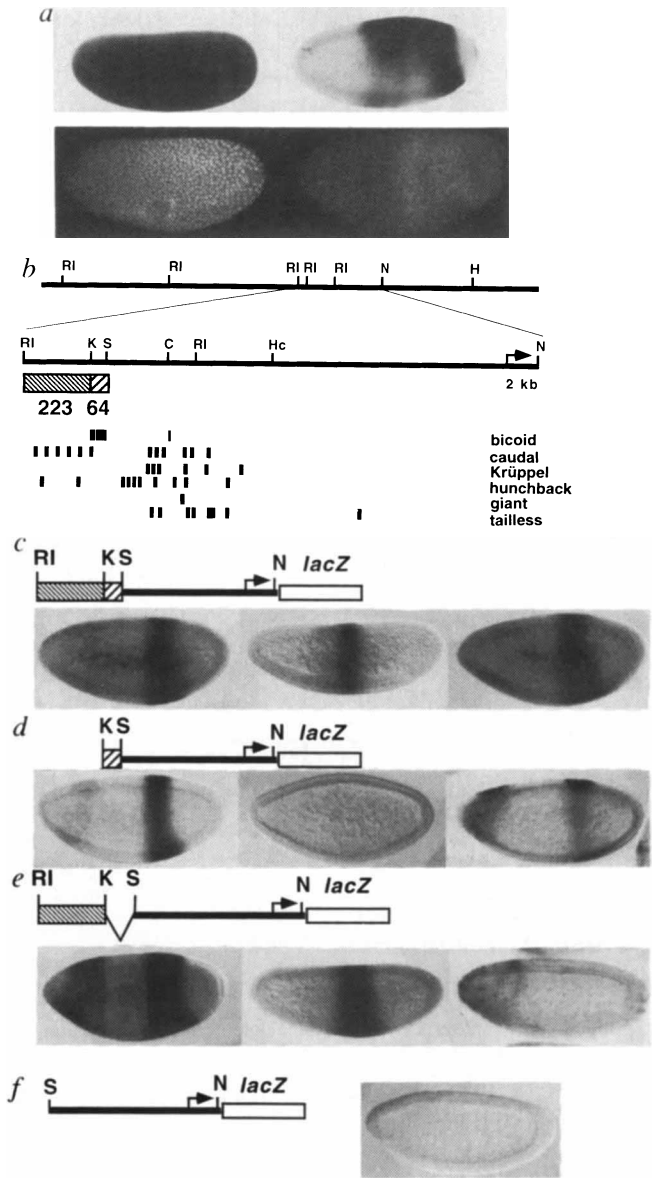

FIG. 1 *kni cis*-acting elements mediating *bicoid*- and *caudal*-dependent activation. *a*, RNA *in situ* hybridization (top) and antibody staining (bottom) showing the expression of the graded maternal (left) and zygotic *caudal* (right). Zygotic *caudal* expression occurs in a broad posterior domain covering the *kni* and *gt* posterior expression domains. Orientation of embryos is anterior left and dorsal up. *b*, Protein binding sites within the 2-kb *kni* control region. Arrow indicates transcription start; the position of the kni223 element ('223') and kni64 element ('64') are shown by narrow- and wide-striped boxes, respectively. Note that the S/Hc fragment mediates *Kr*-dependent enhancing activity and *tll*- and *hb*-dependent repression[7]. RI, *Eco*RI; N, *Nru*I; H, *Hin*dIII; K, *Kpn*I; S, *Sty*I, C, *Cla*I; Hc, *Hin*cII. *c–f*, Reporter gene constructs lacking the kni223 and/or the kni64 element and corresponding expression patterns in transgenic embryos (wild-type embryos are to the left, embryos lacking *bicoid* activity are in the middle, embryos lacking both maternal and zygotic *caudal* activity are to the right). *c*, A transgene containing the kni223 and the kni64 element is expressed in the wild type and in embryos lacking either *bicoid* or *caudal* activity. *d*, A transgene lacking the kni223 element is activated in the wild type and in the absence of *caudal*, but not in the absence of *bicoid*, activity. *e*, A transgene lacking the kni64 element is activated in the wild type and in the absence of *bicoid*, but not in the absence of *caudal* activity. *f*, A transgene lacking both elements is not activated in wild-type embryos. For the orientation of embryos see *a*. Note that an anterior expression domain (*d*, *e*, left and right photograph) is due to promoter sequences of the reporter gene rather than to *kni* sequences; this domain is variable and appears with a frequency of less than 50% with all constructs analysed.

METHODS. RNA *in situ* hybridization with β-galactosidase antisense RNA and antibody stainings were performed as described[5,8]. Antibody-stained embryos (DAPI counterstaining) were examined by laser-scanning microscopy; images were processed by the NIH1.54 software. Transgenic embryos were generated by P-element-mediated transformation[24]; at least two independent transformant lines were analysed for each experiment shown. Reporter gene expression was examined in various mutant embryos (see below) that were produced by bcd^{E1} homozygous females or by cad^{-} germ-line cloned females. cad^{-} germ-line chimaeras were generated by use of the yeast recombinase/dominant female sterility (FLP–DFS) technique[25], which induces site-specific recombination. Females carrying a cad^{2} and FRT recombinant chromosome (genotype: *pr* cad^{2} *P*[*hs-neo*; ry^{+}; *FRT*]^{40A}/*CyO*) were crossed to FRT-DFS males (genotype: *P*[ry^{+}; *hs-FLP*]^{12}; *P*[w^{+}; Ovo^{D1}]$^{2L\text{-}13X13}$ *P*[*hs-neo*; ry^{+}; *FRT*]^{40A}/*CyO*), and the larval progeny heat-shocked at 37 °C. Cy^{+} progeny were mated to males of the genotype *w*; *pr* cad^{3}/*CyO P*[*hb-lacZ*] homozygous for the indicated *kni-lacZ* transgene. Embryos that fail to express the *hb-lacZ* gene allow the distinction between embryos that lack both maternal and zygotic *caudal* activity or maternal *caudal* expression only.

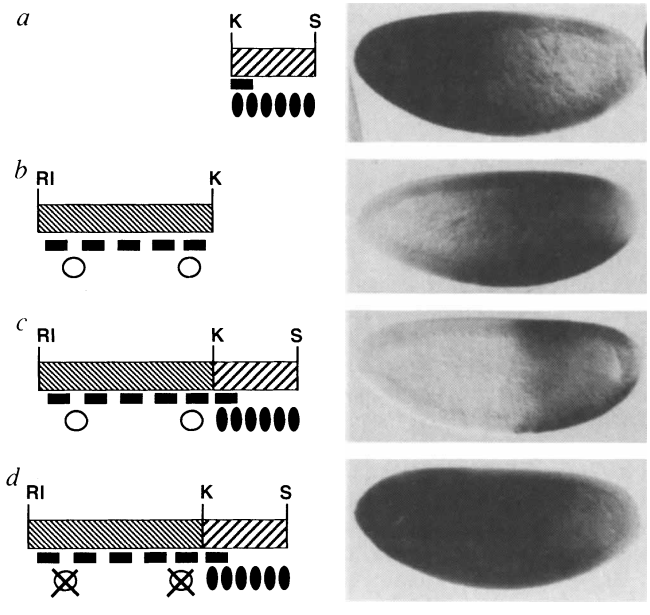

FIG. 2 Combined *bicoid* and *caudal* activities provide activation throughout the embryo. Diagram of kni64 and kni223 elements (left) conducting reporter gene expression patterns as revealed by *in situ* hybridization (right). Orientation of embryos is anterior left and dorsal up; ovals, rectangles and circles refer to bicoid, caudal and hunchback binding sites, respectively (see Figs 1*b* and 3). *a*, The kni64 element conducts *lacZ* expression in an anterior–posterior gradient in wild-type embryos. No expression was observed in embryos lacking *bicoid* activity (results not shown). Note that the single caudal binding site present in the kni64 element does not mediate caudal-dependent activation (see also Fig. 3*h*). *b*, The kni223 element conducts *lacZ* expression reaching about 60% of egg length (0% is the posterior pole). *c*, The combined kni223/kni64 element conducts posterior gene expression similarly to that shown in *b*. *d*, The combined kni223/kni64 element that lacks binding sites for hunchback (Δhbkni223/kni64 element) provides ubiquitous *lacZ* expression (for details see text).

METHODS. Reporter gene constructs contain either the *Eco*RI/*Sty*I fragment (the combined kni223/kni64 element; see Fig. 1), the *Eco*RI/*Asp*718 fragment (kni223 element) or *Asp*718/*Sty*I fragment (kni64 element) fused to the *hs*43 promoter in front of the *lacZ* gene of the pCaSpeR*hs*43 P-element vector[26]. Production of transgenic embryos and *in situ* hybridization are described in Fig. 1. The Δhbkni223/kni64 element was generated by polymerase chain reaction *in vitro* mutagenesis; hunchback fails to bind *in vitro* whereas the overlapped caudal binding site was left unaffected (results not shown).

dominal segmentation pattern. When caudal is absent, the fourth abdominal segment (which is the most sensitive indicator of insufficient *kni* activity[10]) is missing and the other abdominal segments are variably affected[8]. In embryos lacking bicoid activity, maternal caudal is present throughout the embryo[11]. Such embryos develop a normal posterior abdominal segmentation pattern, but their anterior abdominal segment pattern is frequently disturbed[12]. These phenotypes are consistent with the argument that in the absence of either bicoid or caudal, posterior segmentation genes are not expressed at biologically relevant levels. In fact, transcription of *kni* and *gt* is altered in embryos lacking caudal activity (Fig. 4). In the absence of bicoid and of zygotic caudal, *kni* is weakly activated and forms a broad domain in the centre of the embryo (Fig. 4*c*, *f*), whereas *gt* is not expressed (Fig. 4*i*, *l*). In the absence of bicoid and both maternal and zygotic caudal, *kni* is not expressed (results not shown). Furthermore, because caudal functions as a transcriptional activator in transfected tissue culture cells[13] and is able to activate posterior stripe expression of the pair-rule genes *fushi tarazu* (*ftz*)[13] and *hairy* (unpublished results), the combined maternal and zygotic caudal activities may function as posterior activator at different levels of the segmentation gene cascade. The partial complementation of the lack of caudal function by bicoid (Fig. 4*b*, *e*, *h*) explains why the major role of caudal as an activator of posterior patterning genes had escaped discovery by formal genetic studies.

Our results establish that posterior gap genes are activated in response to the combined activities of two roughly reciprocal transcription factor gradients to simultaneously blueprint the segment equivalents along the anterior–posterior axis of the *Drosophila* preblastoderm[1,2] and to generate all segments at the same time during gastrulation. The sequential generation of abdominal segments within an initially uncommitted cellular growth zone of the gastrula[14] of more primitive insects was taken to speculate that the molecular system acting in basic *Drosophila* segmentation may not function within the cellular context[15,16]. This assumption was supported by the lack of success in identifying *bicoid* homologues in insects other than higher dipterans.

In contrast to *bicoid*, *caudal* homologues are known among various species, and they exert graded expression patterns in a cellular context even in vertebrates[17–19]. Furthermore, a *Xenopus* gene with sequence similarity to *nanos*, the decisive component of the maternal posterior system[1,2] of *Drosophila*, has been isolated[20] and *glp*-RNA of the nematode *Caenorhabditis elegans*, which is under translational control by a hypothetical regulator that may be restricted to the posterior cell, has been shown to contain a *nanos*-response element[21]. These observations and the isolation of *hunchback*-like genes in several arthropods[22] suggest a conserved model where *nanos* activity eliminates *hunchback* activity from the posterior precellular blastoderm region of more primitive insects; zygotic caudal could then function there at a later cellular stage. In fact, a corresponding cellular gradient

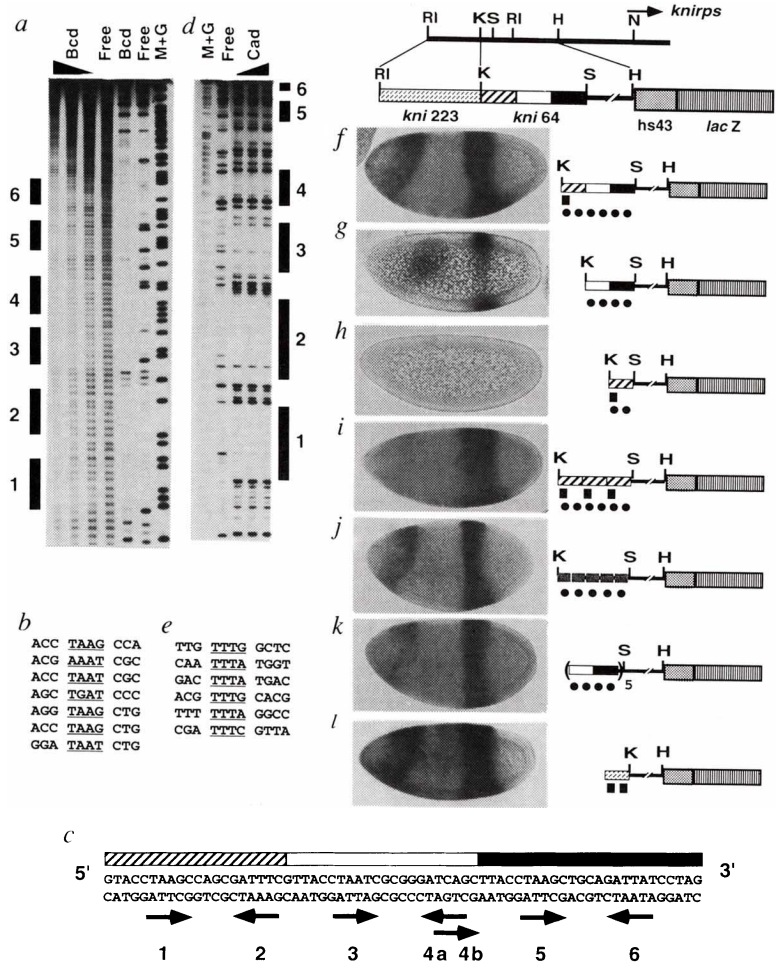

FIG. 3 Molecular organization of the kni223 and kni64 elements and posterior gene activation in response to *bicoid* and *caudal*. *a–c*, kni64 element DNA contains six binding sites for bicoid *in vitro* as revealed by DNase I and hydroxyl radical footprinting (*a*). A + G, Maxam–Gilbert reaction; Free, control reaction with kni64-element DNA; Bcd: reaction with kni64-element DNA and recombinant bicoid. Bars indicate protected sites. *b*, Bicoid binding sites (see *d*) represent 'weak sites' according to the established consensus[27]. Underlined sequences represent the core consensus. *c*, Sequence and diagram of binding sites derived from hydroxyl radical footprinting; orientation of binding sites is indicated by arrows. Note that site 4 can be covered in both orientations and that the sites are arranged in three pairs of quasi-palindromic sequences. Contacted nucleotides are 10–11 bp as determined by DMS protection (R.R.-P., unpublished). *d*, The kni223 element contains six caudal binding sites of variable affinities as determined by quantitative DNase I footprinting (R.R.-P., results not shown). *e*, Consensus sequences of caudal sites; core is underlined. *f–l*, Reporter gene expression (left) and corresponding transgene constructs (right). Circles: bicoid sites; rectangles: caudal sites. Note that the transgenes shown in *f–k* lack the kni223 element but contain either the kni64 element (*f*; see also Fig. 1*d*) or modified kni64 elements (*g–k*) in the context of the otherwise intact *kni cis*-acting control region (details in Fig. 1*b*); modifications are deletions or new combinations of bicoid binding sites (coded by bars according to *c*). Four bicoid sites are sufficient for *bicoid*-dependent gene activation (*g*), whereas two bicoid binding sites are not (*h*). *bicoid*-dependent activation is independent of actual binding site sequences, because a tandem repeat of three copies of the 5′ 20-bp sequence of the kni64 element (sites 1, 2; see *c*) causes posterior gene expression (*i*) as do five copies of site 5 (*j*). An excess of bicoid sites (five tandem arrays of sites 3–6; see *c*) does not affect the size of the posterior expression domain (*k*). Two high-affinity caudal sites (sites 2, 3) are sufficient for gene activation in the absence of kni64 element (*l*).
METHODS. DNase I[28] and hydroxyl radical[29] footprinting were performed with a bacterially expressed and purified truncated bicoid (expanding from amino acid 89 to 154) or caudal protein (amino acids 58–377) and the autoradiographs were quantified either by phosphor imaging or densitometry. Other techniques are described in Figs 1 and 2.

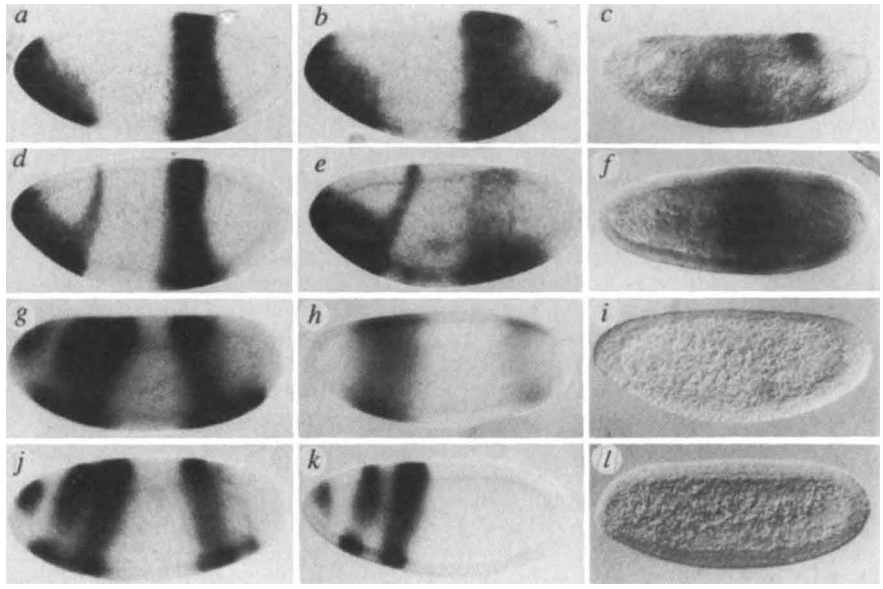

FIG. 4 Posterior gap gene expression directed by bicoid and caudal. *In situ* hybridization showing *kni* (*a–f*) and *gt* (*g–l*) expression in wild-type embryos (*a, d, g, j*), in embryos lacking maternal and zygotic *caudal* activity (*b, e, h, k*) and in embryos lacking *bicoid* and zygotic *caudal* (*c, f, i, l*). Orientation of embryos is anterior left, dorsal up. In the absence of both maternal and zygotic *caudal*, *kni* posterior expression is reduced and expands posteriorly at syncytial blastoderm stage (compare *a, b* and *d, e*). The posterior expansion of *kni* expression (*b, e*) might be due to the absence or reduction of a caudal-dependent posterior *kni* repressor such as *gt* (ref. 2) (see below). At cellular blastoderm stage (*d, e*) both effects are more pronounced. In the absence of *bicoid* and zygotic *caudal* activity, *kni* is seen only after prolonged staining reactions (*c, f*); owing to the lack of repressor activities (zygotic hunchback[5,7] and *gt* (ref. 30); see *i, l*), weak, *kni* expression covers a broad zone. This indicates that maternal *caudal* activity activates *kni* only weakly when bicoid is absent (*c, f*). *g, h*, Reduction of posterior *gt* expression in embryos lacking maternal and zygotic *caudal* (compare *g, h* and *j, k*) is more pronounced than with *kni* (compare *b, h* and *e, k*). *j, k*, The posterior *gt* domain is absent in embryos lacking *caudal* activity at the cellular blastoderm stage. The low level of initial posterior *gt* expression at syncytial blastoderm stage indicates activation independent of *caudal* activity likely to be provided by bicoid (*i, l*). METHODS. Whole-mount *in situ* hybridization using digoxigenin-labelled *kni* and *gt* antisense RNA and the generation of *cad* mutant embryos and their identification are described in Fig. 1 legend.

of caudal has recently been observed in *Bombyx mori*[23]. While forming, the gradient of caudal could provide distinct threshold concentrations to control members of the three-tiered segmentation-gene cascade in space and time, that is, the gradient of caudal could provide the basis for asynchronous segment development in primitive insects by heterochronic gene activation. This role could be analogous to that of the early expression of caudal in vertebrates[17–19], and possibly represents a widely conserved feature of early development. Zygotic *caudal* could therefore represent the ancient function. In this view, its maternal complement and the ability of *bicoid* to support *caudal* function in the posterior region of the *Drosophila* blastoderm might be a synapomorphy for higher dipterans to adopt a rapid patterning system that acts in a synchronous fashion during early gastrulation. □

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Presynaptic changes during mossy fibre LTP revealed by NMDA receptor-mediated synaptic responses

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ACTIVITY-DEPENDENT changes in synaptic strength are important for learning and memory. Long-term potentiation (LTP) of glutamatergic excitatory synapses following brief repetitive stimulation provides a compelling cellular model for such plasticity[1–4]. In the CA1 region of the hippocampus, anatomical studies have revealed large numbers of NMDA (*N*-methyl-D-aspartate) receptor sites at excitatory synapses[5,6], which express primarily an NMDA receptor-dependent form of LTP[7]. In contrast, these studies[5,6] have suggested that mossy fibre synapses activate primarily or exclusively α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and, indeed, these synapses express a form of LTP that is entirely independent of NMDA receptors[8,9]. Here we present physiological data demonstrating that mossy fibres activate a substantial NMDA receptor synaptic component that expresses

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