tandem repeats from the CA6 NOR in parthenogens with both SM6 and CA6 rDNA. If biased unequal crossing over is operating, most tandem repeats should be of one parental sequence, whereas biased gene conversion should produce interspersed mixtures of both sequences. In any case, there is strong, unidirectional bias in the concerted evolution of *Heteronotia* rDNA arrays in favor of the SM6 genotype.

The processes responsible for concerted evolution may differ between parthenogenetic and sexual species. Unequal crossing over could be restricted in parthenogens because of the requisite modifications to meiosis in these species (*18*). However, this study demonstrates that concerted evolution of rDNA can be driven by directional, rather than stochastic, processes, and that these directional processes do occur among (as well as within) chromosomes. It also supports the idea that some mutations in repeated gene families may spread rapidly through the genome ("molecular drive") (*4*), even in supposedly clonal organisms such as parthenogens. Finally, the concept of a "permanent hybrid" genome in parthenogenetic vertebrates (*9*) does not apply to repeated DNA sequences, which continue to evolve in a concerted fashion in these species.

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11. Methods as described in (7).
12. The individual represented in lane 6 of Fig. 2 has the rDNA restriction patterns of all three sexual species. However, analysis of chromosomes and allozymes did not reveal any of the other markers diagnostic for the EA6 sexuals.
13. SM6 rDNA is expected to be more abundant in the triploids with two doses of the SM6 genome, even in the absence of biased gene conversion. If the comparison is restricted to the 2CA6/1SM6 individuals, in which CA6 rDNA should be approximately twice as abundant as SM6 rDNA if biased conversion does not occur, the SM6 rDNA was more abundant than the CA6 fragment in 36 of 41 individuals. In the single individual with all three diagnostic fragments, the relative concentration of the three fragments was EA6 > SM6 > CA6.
14. Silver staining was modified from C. Goodpasture and S. E. Bloom, *Chromosoma* **53**, 37 (1975). Approximately 200 μl of 2% gelatin (with 1% formic acid) was mixed with an equal volume of 50% silver nitrate on each slide. Slides were incubated for 6 to 9 min at 37°C, rinsed for 4 min in 5% sodium thiosulfate, and lightly counterstained in 2% Giemsa.
15. We performed in situ hybridization using as probe the I-19 rDNA cloned from *Mus musculus* by N. Arnheim, *Gene* **7**, 83 (1979). The plasmid was biotin-labeled, and hybridization followed the procedure in R. K. Moyzis *et al.*, *Chromosoma* **95**, 375 (1987), except that we used blaze-dried chromosome spreads to better visualize centromere position.
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The Effect of the Floor Plate on Pattern and Polarity in the Developing Central Nervous System

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The effect of floor plate on cellular differentiation in the neural tube of quail embryos was examined. In the developing neural tube the floor plate, which consists of specialized neuroepithelial cells, is located in the ventral midline of the neural tube. When Hensen's node was extirpated the floor plate and notochord did not develop, and the normal differentiation of the ventral horn motor neurons and dorsal and ventral roots did not occur. When one side of the neural tube was deprived of notochord, the ventro-dorsal differentiation took place on both sides. However, when one side of the neural tube was deprived of the floor plate, the ventral horn motor neurons and dorsal and ventral roots did not develop on that side. These observations suggest that the floor plate influences motor neuron differentiation and acts as an intrinsic organizer to establish pattern and polarity in the developing nervous system.

SHORTLY AFTER THE CLOSURE OF THE neural tube, functionally distinct classes of neurons differentiate in specific locations within the neural tube (*1*). For example, motor neurons begin to differentiate in the ventral horn of the spinal cord and send their axons out by way of the ventral root. Subsequently, sensory neurons develop in the dorsal horn and sensory nerve fibers from the periphery enter the central nervous system (CNS) through the dorsal root. It is not clear which factors establish this basic pattern. Recent studies indicate that in rats the floor plate provides chemo-

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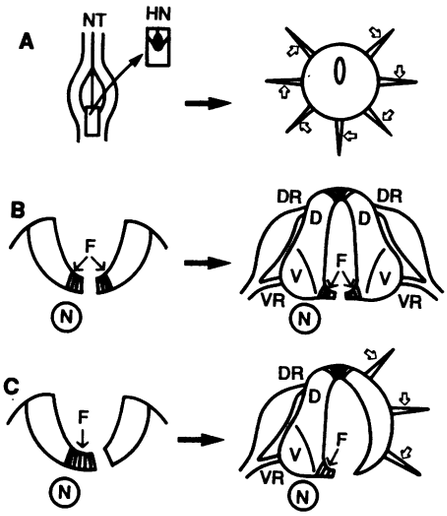

Fig 1. Schematic representation of the three types of surgical operations on Japanese quail embryos and their results. (**A**) Hensen's node removal at stage 10; (**B**) median section of floor plate at stage 12 to 13; (**C**) paramedian section of floor plate at stage 12 to 13 (*10*). The rostro-caudal extent of the incisions in (B) and (C) ranged from the region of the last-formed somite (15th somite) to the region just anterior to Hensen's node. This distance corresponds to about ten somites in length in the lumbar region. The depth of the incision was all the way through the endoderm. A total of 614 embryos was operated on. Out of 147 embryos in which Hensen's node was removed, 63 were fixed; out of 55 embryos with median section of floor plate, 37 were fixed; and out of 412 embryos with paramedian section of floor plate, 346 were fixed (*11*). Embryos were fixed and processed either for histology or for Golgi staining (*12*). D, dorsal horn; DR, dorsal root; F, floor plate; HN, Hensen's node, N, notochord, NT, neural tube; V, ventral horn; VR, ventral root. Open arrows indicate ectopic fibers.

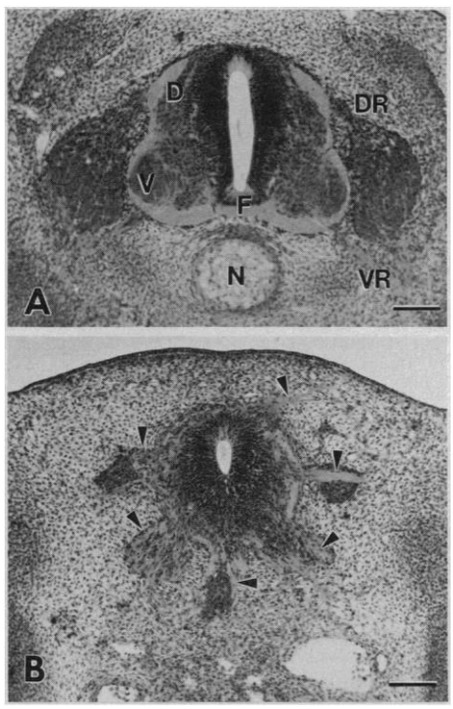

Fig 2. (**A**) Cross section through the lumbar region of a normal stage-25 quail embryo showing differentiation of ventral horn (V), dorsal horn (D), ventral root (VR), and dorsal root (DR). F, floor plate; N, notochord. (**B**) Cross section through the lumbar region of a stage-25 quail embryo after early removal of Hensen's node shows disorganized spinal cord. Floor plate and the notochord are absent. The ventro-dorsal differentiation did not occur. Ectopic nerve fibers (arrowheads) radiated from various regions of the spinal cord. Bars represent 100 μm.

tactic guidance to axons that cross to the opposite side of the spinal cord through the ventral commissure (*2*). It has been suggested that the floor plate may also contribute to other aspects of pattern and polarity in the developing spinal cord (*3*).

Hensen's node, a group of cells located at the rostral tip of the primitive groove, gives rise to the notochord. Both structures together contribute to the formation of the floor plate (*4*). Hensen's node was surgically extirpated in the thoracic region of Japanese quail embryos at stage 10 (*5*) in order to prevent the formation of the notochord and the floor plate in the caudal spinal cord (Fig. 1A). Spinal cords were examined histologically at stage 25 (embryonic day 5). Normally, by this time the ventral horn can be distinguished from the dorsal horn by virtue of the differentiation of hindlimb motor neurons in the lateral motor column, which produces a ventrolateral bulge in the spinal cord; the ventral and dorsal roots are also well developed (Fig. 2A). Extirpation of Hensen's node resulted in absence of the notochord and the floor plate (Fig. 2B). Although the neural tube closed, it was misshapen and disorganized, and cells of the ventral horn did not correctly differentiate into motor neurons on either side. Nerve fibers radiated from multiple sites instead of through the dorsal and ventral roots (Fig. 2B). These observations indicate that the presence of the notochord and the floor plate is necessary for the normal development of the basic pattern in the spinal cord.

Whether the disorganization of the spinal cord after Hensen's node elimination was due to absence of the notochord was subsequently investigated. A median incision was made in the floor plate of the lumbar region prior to neural tube closure (stage 12 to 13) (Fig. 1B). The neural tube closes in the more rostral regions at stage 10 to 11 and in the caudal regions at about stage 13 to 14. The incision was made in such a way that the notochord was shifted entirely to one side, whereas half of the floor plate was present on each side (Fig. 3A). Both sides of the spinal cord differentiated normally. This observation suggests that the presence of notochord adjacent to each half of the neural tube is not necessary for the development of the characteristic pattern in the spinal cord.

Whether the floor plate was responsible for the basic organization of the spinal cord was then investigated. A paramedian incision in the floor plate was made so that one side of the neural tube was deprived of its floor plate (Fig. 1C). This type of operation resulted in failure of normal differentiation of the ventro-dorsal polarity in the spinal cord only on the side lacking floor plate (Fig. 3B). The ventral horn, which contains

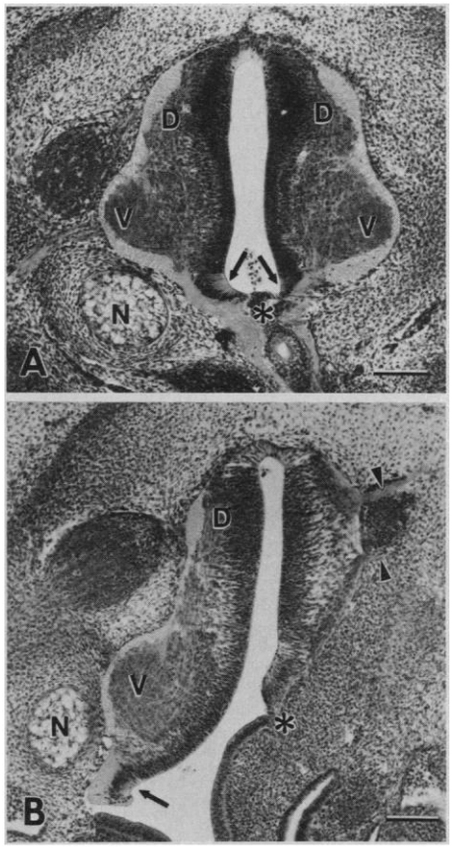

Fig 3. (**A**) Cross section through the lumbar region of a stage-25 quail embryo after median section of the floor plate. The notochord (N) shifted to one side whereas half of the floor plate (arrows) is present on each side. The ventral horn (V) can be easily distinguished from the dorsal horn (D) on the side lacking notochord. Asterisk indicates fusion of neuroepithelium and endoderm. In some cases the two epithelia were separated by mesenchymal cells. (**B**) Cross section through the lumbar region of a stage-25 quail embryo following paramedian section of floor plate. The floor plate (arrow) is present only on the left side. The ventral horn (V) and dorsal horn (D) differentiated normally on the side with the intact floor plate (arrow), but not on the side lacking the floor plate. Asterisk indicates the point at which neuroepithelial cells on the side of the spinal cord lacking floor plate fused with the endodermal epithelial cells. The neural tissue just dorsal to the asterisk is not floor plate as it lacks not only the marginal zone but also cells with abluminal location of their elongated nuclei. N, notochord. Arrowheads point to the ectopic nerve fibers. Bars represent 100 μm.

motor neurons, did not develop on the affected side. Nerve fibers radiated from multiple sites rather than through the usual dorsal and ventral roots. These nerve fibers had their cell bodies located in the dorsal part of the spinal cord (Fig. 4A), projected to muscles (Figure 4B), and caused muscle contractions (*6*). Because neither the alar plate nor the basal plate was damaged by the surgical procedure (Fig. 4C), it is unlikely that this arrangement was due to the disappearance of the dorsal horn and subsequent upward displacement of the ventral horn. Normally, commissural neurons in the dorsal horn project their axons ventrally. However, under these experimental conditions those neurons act like motor neurons in that their axons leave the CNS and functionally innervate muscles. It is quite likely that the lack of a chemoattractant from the floor plate (*2*) may be responsible for the motor neuron–like behavior of these commissural fibers.

Although our results suggest that the presence of floor plate is essential for the normal differentiation of ventral horn motor neurons and the establishment of pattern and polarity in the developing neural tube, they do not reveal the underlying mechanism. It seems reasonable that the floor plate may first specify the differentiation of neurons located adjacent to it to become motor neurons. Then the more distant, dorsally located neuronal populations would be specified to become sensory (association and commissural) neurons. This would imply that neurons located in dorsal regions of the neural tube are capable of becoming motor neurons and that they are normally inhibited from doing so either by some direct influence of the floor plate on them or by the influence of differentiated motor neurons in the ventral horn. Several lines of evidence are consistent with this view. First, the floor plate develops prior to the onset of differentiation of neurons and thus is in a position to exert an influence on them. Second, the differentiation of motor neurons precedes that of sensory neurons and it is the motor neurons that are located adjacent to the floor plate (*1*). Third, in the absence of floor plate, the motor neurons in the ventral horn do not develop. Instead, neurons located in the dorsal part of the neural tube act like motor neurons (Figs. 3 and 4). The floor plate could accomplish both the specification of nearby cells to become motor neurons and the inhibition of more distal cells from becoming motor neurons through secretion of diffusible substances, possibly retinoids, which would form a ventro-dorsal gradient through the neural tube.

Floor plate can synthesize retinoids and when grafted to the anterior part of chick limb it can specify formation of additional digits (*7*), an effect similar to that achieved by local application of retinoic acid (*8*). Two intracellular proteins which are thought to be involved in retinoid-mediated signal transduction pathways show a non-overlapping, ventro-dorsal, distribution in the developing nervous system. Cellular retinol binding protein is present in the floor plate and in cells which will later differentiate into ventral horn motor neurons whereas cellular retinoic acid binding protein is distributed in the region of the dorsal horn of the developing neural tube (*9*).

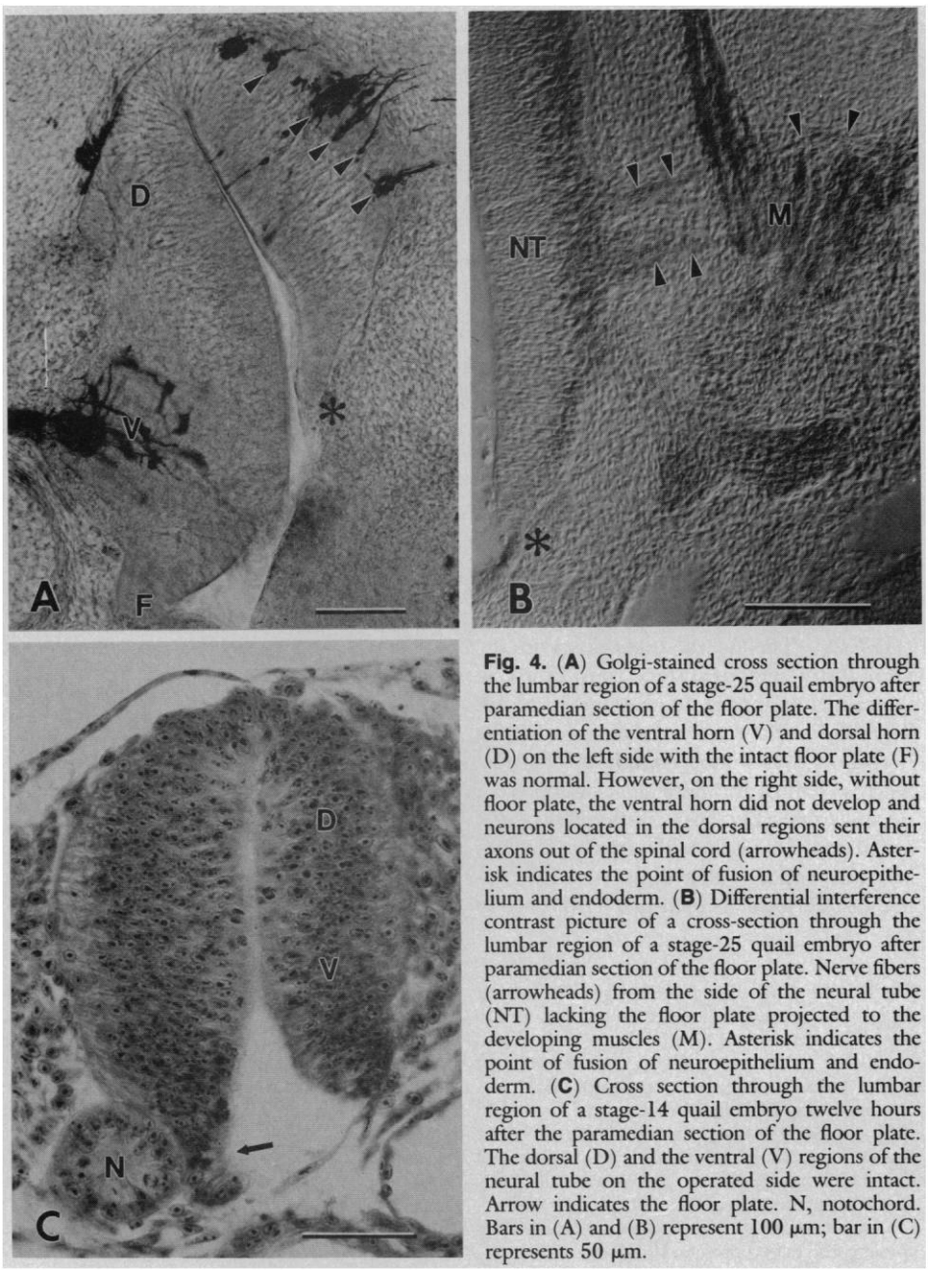

Fig. 4. (**A**) Golgi-stained cross section through the lumbar region of a stage-25 quail embryo after paramedian section of the floor plate. The differentiation of the ventral horn (V) and dorsal horn (D) on the left side with the intact floor plate (F) was normal. However, on the right side, without floor plate, the ventral horn did not develop and neurons located in the dorsal regions sent their axons out of the spinal cord (arrowheads). Asterisk indicates the point of fusion of neuroepithelium and endoderm. (**B**) Differential interference contrast picture of a cross-section through the lumbar region of a stage-25 quail embryo after paramedian section of the floor plate. Nerve fibers (arrowheads) from the side of the neural tube (NT) lacking the floor plate projected to the developing muscles (M). Asterisk indicates the point of fusion of neuroepithelium and endoderm. (**C**) Cross section through the lumbar region of a stage-14 quail embryo twelve hours after the paramedian section of the floor plate. The dorsal (D) and the ventral (V) regions of the neural tube on the operated side were intact. Arrow indicates the floor plate. N, notochord. Bars in (A) and (B) represent 100 μm; bar in (C) represents 50 μm.

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10. Eggs of Japanese quail embryos were incubated at 37.5°C and in a humidity of about 90%. On the second day of incubation, a window was made in the egg shell and India ink was injected beneath the embryo for visualization. Hensen's node was cut with a fine stainless steel needle at stage 10 and removed with a micropipette. Median and paramedian incisions of the floor plate were also made with a fine needle at stage 12 to 13. All microsurgery was performed under aseptic conditions. After surgery the egg shell window was covered with Parafilm and embryos were reincubated and killed on embryonic day 5.
11. The results presented here were based on observations of a majority of these embryos. Some embryos turned out to be unsuitable for the study because of incomplete operations, retarded growth, and gross malformations.
12. For histology, embryos were fixed in Bouin's solution, embedded in paraffin, serially sectioned at 15 μm thickness, and stained with hematoxylin and eosin. For Golgi staining, embryos were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and processed for routine rapid Golgi impregnation.
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Regulation of Interleukin-2 Gene Enhancer Activity by the T Cell Accessory Molecule CD28

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The mechanism by which cell surface molecules regulate T cell production of lymphokines is poorly understood. Production of interleukin-2 (IL-2) can be regulated by signal transduction pathways distinct from those induced by the T cell antigen receptor. Stimulation of CD28, a molecule expressed on most human T cells, induced the formation of a protein complex that bound to a site on the *IL-2* gene distinct from previously described binding sites and increased *IL-2* enhancer activity fivefold. The CD28-responsive complex bound to the *IL-2* gene between −164 and −154 base pairs from the transcription start site. The sequence of this element is similar to regions conserved in the 5′ flanking regions of several other lymphokine genes.

T LYMPHOCYTES HAVE AN IMPORTANT role in most immunological responses. To exert their effector functions, relatively quiescent T cells are activated during a complex interaction with antigen-presenting cells. The specificity of a T cell response to antigen is mediated by the T cell antigen receptor (TCR) complex, which contains a ligand binding subunit, the immunoglobulin-like heterodimer Ti, noncovalently associated with at least five invariant proteins (*1*). Interaction of the TCR with antigen in association with the major histocompatibility complex (MHC) or antibodies to the TCR results in the initiation of signal transduction events and cellular activation. Perturbation of the TCR activates a tyrosine kinase and phospholipase C, which results in tyrosine phosphorylation and phosphatidylinositol 4,5-biphosphate hydrolysis, respectively (*2*). These events are thought to lead to a variety of cellular responses, one of which is lymphokine production.

However, stimulation of the TCR alone is insufficient to activate most T cells. In addition to the TCR, a number of other T cell surface molecules appear to contribute to T cell activation and proliferation (*3*). One of these, CD28, is a 44-kD glycoprotein that is expressed as a homodimer on the majority of human T cells (*4*). In T cells activated via their TCR or by pharmacological agents that mimic TCR-induced phospholipase C activation, the addition of antibody to CD28 (anti-CD28) causes a marked increase in the mRNA and secretion of several T cell lymphokines, including interleukin-2 (IL-2), tumor necrosis factor–α, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), and lymphotoxin (*5*). The nature of the CD28-generated transmembrane signal in response anti-CD28 treatment is unclear, but is probably distinct from those of the T cell antigen receptor (*6*).

The induction of *IL-2* gene transcription during T cell activation is mediated primarily by a region extending 326 bp upstream of the transcription start site (*7*). This region contains binding sites for several nuclear proteins (*8, 9*) (Fig. 1). To investigate whether CD28 stimulation affects *IL-2* gene transcription, we tested constructs that contain this region of the *IL-2* gene linked to the reporter genes luciferase (Luc) or chloramphenicol acetyltransferase (CAT) in transient expression studies. In contrast to an earlier report that showed an increased stability of *IL-2* message but did not detect an effect on *IL-2* transcription (*10*), our studies indicate that CD28 stimulation caused a significant increase in *IL-2* enhancer activity. We have identified a previously uncharacterized element within the *IL-2* enhancer that is a target sequence for a CD28-regulated nuclear binding complex.

The plasmid pIL-2–Luc, a recombinant reporter plasmid that contains sequences from −326 to +46 of the human *IL-2* gene directing transcription of the firefly luciferase gene (*8*), was transfected into the Jurkat T cell line. The transfected cells were then treated for 8 hours with combinations of ionomycin and phorbol 12-myristate 13-acetate (PMA), agents that mimic the effect of TCR-induced phospholipase C activation by increasing the cytoplasmic Ca^{2+} concentration and activating protein kinase C, respectively. The transfected cells were stimulated in the presence or absence of monoclonal antibodies (MAbs) to either CD28 or class 1 histocompatibility antigens

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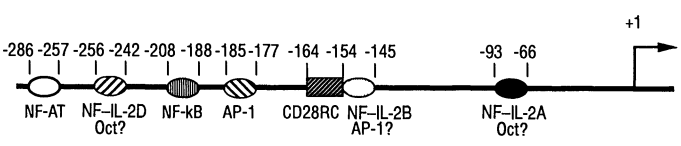

Fig. 1. The *IL-2* enhancer with known protein binding sites. The numbers at the top of the figure represent the position in base pairs relative to the initiation of transcription site.