suppressive effect of **2** was similar, from 15 to 120 minutes (the longest time tested) after administration, whereas that of **4** showed a time course similar to other dopaminergic agents such as apomorphine. Apomorphine given at a similar dose level (1 mg per kilogram of body weight, intravenously) to estrogen-primed female rats reduced serum prolactin by 50 percent at 30 minutes but was without effect at 60 minutes (*11*). Thus, the brief activities of apomorphine and of **4** seem to be due to their rapid clearance from the body. In the case of **4**, however, dopamine formed at the anterior pituitary could still be responsible for the transient reduction in prolactin secretion. To evaluate this possibility, we compared the activity of **4** in vitro with that of dopamine (**1**) (Fig. 3). If it is active at all, compound **4** is considerably less active than **1**. The lack of activity by **4** at 2×10^{-7} *M* also indicates that metabolism to **1** does not take place to any significant extent in the media used for testing in vitro.

Thus, we have succeeded in producing significant and sustained brain-specific dopaminergic activity by a mechanism of sustained local release of dopamine from the locked-in delivery form. This delivery system may be useful in the treatment of parkinsonism and hyperprolactinemia, and similar methods could be used for the selective delivery of other neurotransmitters to the brain.

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Transmission of Integrated Sea Urchin Histone Genes by Nuclear Transplantation in *Xenopus laevis*

Abstract. *Sea urchin histone genes contained in a recombinant plasmid pSp102 were microinjected into the cytoplasm of fertilized eggs of* Xenopus laevis. *By the late blastula stage, plasmid DNA sequences were detected comigrating with the high molecular weight cellular DNA (greater than 48 kilobases). Analysis of the DNA from injected embryos digested with various restriction endonuclease demonstrated that the injected DNA was integrated into the frog genome. Clones of embryos containing the pSp102 DNA sequences were produced by means of nuclear transplantation. Individuals of the same clone contain the pSp102 sequences integrated into similar chromosomal locations. These sites vary between different clones.*

Recently the developmental and tissue specific expression of individual cloned genes has been examined by microinjection into the fertilized eggs of amphibians, mammals, and insects (*1–11*) [for review, see (*12–14*)]. During early developmental stages in the frog the injected circular DNA sequences migrate on agarose gels both as the circular plasmid DNA and as high molecular weight cellular DNA, and appear to replicate (*1–3*). In most cases, after the gastrula stage of development, the circular plasmid sequences are no longer detected (*1–3*). In the mouse and fruit fly the injected genes are integrated and the integrated DNA sequences are transmitted in the germ line to the next generation (*4, 7, 8, 10, 11*). Injected DNA sequences are expressed during early development (*1–3*) and in adult tissues (*4–10*) but not in a predictable manner.

The aberrant expression of injected DNA's may be due to expression of nonintegrated sequences during early development or the integration of exogenous genes into various locations in the host chromosome. Therefore, even though the injected genes contain the DNA sequences necessary for transcription (that is, CCAAT and TATA; C, cytosine; A, adenine; T, thymine) located at their 5′ end, their developmental or tissue specific expression may be affected by the absence of other sequences that normally surround the gene in the chromosome.

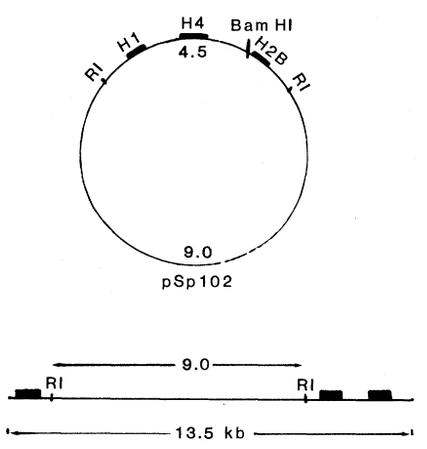

Fig. 1. Diagram of Bam HI and Eco RI restriction sites in plasmid pSp102. Treatment of plasmid DNA with Bam HI produces a 13.5-kb linear DNA fragment. There are no Hind III sites in the plasmid.

We now report that sea urchin histone genes are integrated into the frog genome after microinjection into the cytoplasm of fertilized eggs of *Xenopus laevis*, and that they can be transmitted to clones of frogs by nuclear transplantation. This procedure permits the analysis of developmental and tissue-specific expression of injected genes in clones of frogs in which the gene is integrated into a specific chromosomal location, thus enabling us to assess the effect of chromosomal location on the expression of injected genes.

Copies (10^4 to 10^6) of pSp102 (*15*) containing the sea urchin histone genes H1, H4, and H2B in Col El were injected into the cytoplasm of each egg within 1 hour after the eggs were artificially inseminated. High molecular weight DNA was extracted from embryos and larvae with phenol, chloroform, and isoamyl alcohol. The DNA was separated by electrophoresis on 0.7 percent agarose gels and transferred to nitrocellulose filters (*16*). The blots were hybridized with a nick-translated probe prepared from pSp102 (*17, 18*). The pSp102 DNA sequences were detectable in 90 percent of the injected blastulas (*3*). During early cleavage stages most of the DNA comigrated with circular plasmid DNA molecules (that is, either form I, supercoiled, or form I^r, relaxed, and form II). By the late blastula stage a portion of the exogenous DNA comigrated with the high molecular weight cellular DNA in approximately 20 to 30 percent of the injected embryos. After the blastula stage, there was a gradual loss of the circular molecules until few, if any, circular DNA molecules were detectable by the late tail bud stage [stages 29 and 30 (*19*)] (*1–3*).

Integration of exogenous DNA sequences was shown by digestion of DNA

from injected embryos with several restriction enzymes that cut either once or not at all within the plasmid sequences (Fig. 1) (*15*). Digestion of the plasmid pSp102 with restriction endonuclease Bam HI produced DNA fragments, differing in size from the expected 13.5-kb linear pSp102 plasmid (Figs. 2A and 3B). These represent putative junction fragments consisting of frog DNA joined to pSp102 sequences. Bam HI digestion of DNA from embryos at the blastula stage of development produced the linear 13.5-kb DNA fragments resulting from circular pSp102 plasmids (Fig. 2A, lanes 2 and 7). DNA fragments not predicted from the restriction map of pSp102 were also observed. Lane 2 shows the presence of two putative junction fragments of approximately 6.6 and 17 kb. Lane 4 shows an analysis of a mixture of DNA's from 15 injected embryos (Fig. 2, lane 4).

A second restriction enzyme, Hind III, which does not cleave the plasmid pSp102 was also used. Treatment of DNA from injected embryos and larvae produced bands that were not predicted from the restriction map of the plasmid pSp102 (Fig. 2B). Hind III digestion of the DNA of tail muscle (lane 1) and heart (lane 3) from an 8-week-old larva produced a band approximately 20 to 22 kb in size. This band was smaller in size than pSp102 sequences from undigsted DNA (Fig. 3A, lane 1) or from partially digested (Hind III) DNA from the gut of the same animal (Fig. 2B, lane 2). These data in Fig. 2 are consistent with the hypothesis that the injected pSp102 DNA sequences were integrated into the frog genome after injection into the cytoplasm of the fertilized egg. We estimate that approximately one to ten copies of the plasmid were integrated into the genome of the larvae which were analyzed (*3*). We have been able to transmit integrated exogenous DNA sequences to clones of embryos by means of nuclear transplantation. This involved transferring individual nuclei (as well as cytoplasm) from transformed gastrulas into activated nucleated eggs (*20*). DNA was extracted from recipient embryos and either digested with restriction enzymes or analyzed undigested.

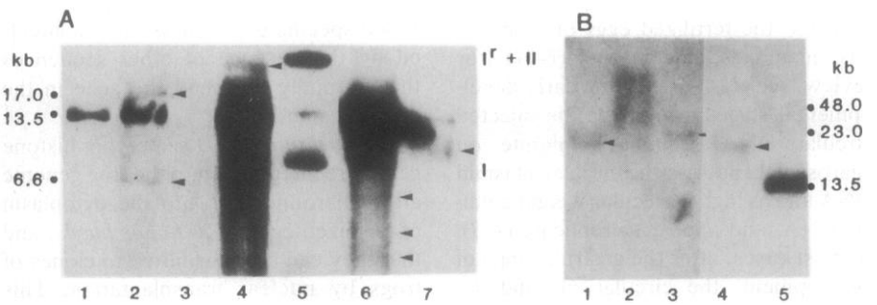

Fig. 2. (A) Autoradiograph of DNA treated with Bam HI (cuts pSp102 at a single site) from injected embryos at the blastula stage. The plasmid pSp102 was injected within 1 hour after artificial insemination; DNA was extracted at the blastula stage, digested with Bam HI, subjected to electrophoresis on 1 percent agarose gels, blotted to nitrocellulose, and hybridized with a nick-translated probe produced from pSp102. (Lane 1) Plasmid pSp102 mixed with noninjected frog DNA and then digested with Bam HI, showing the presence of a single linear 13.5-kb DNA fragment; (lane 2) DNA from embryos (two) treated with Bam HI showing the presence of two putative junction fragments (arrows); (lane 3) DNA from noninjected *Xenopus* embryos (two embryo equivalents); (lane 4) A portion of DNA from 15 injected embryos treated with Bam HI, showing smear of junction fragments (arrow points to one fragment); (lane 5) marker pSp102 mixed with *Xenopus* embryo DNA showing the positions of form I^r (covalently closed circular relaxed molecules), form I (covalently closed supercoiled), form III (13.5-kb linear DNA molecules), and form II (nicked relaxed circular); (lane 6) Bam HI digested DNA from a single injected embryo showing many different junction fragments; (lane 7) Bam HI treated DNA from a single blastula embryo showing a different pattern of fragments (arrow). (B) Autoradiograph of Hind III digests of DNA from injected neurula and tissues of 8-week-old larva. (Lane 1) Hind III digest of tail muscle tissue DNA of 8 week-old larva; (lane 2) partial Hind III digest of gut DNA from the same larva in lane 1; (lane 3) Hind III digest of DNA from heart muscle of same larva; (lane 4) Hind III digest of DNA from injected neurula stage embryo; (lane 5) marker 13.5-kb linear DNA fragment of pSp102.

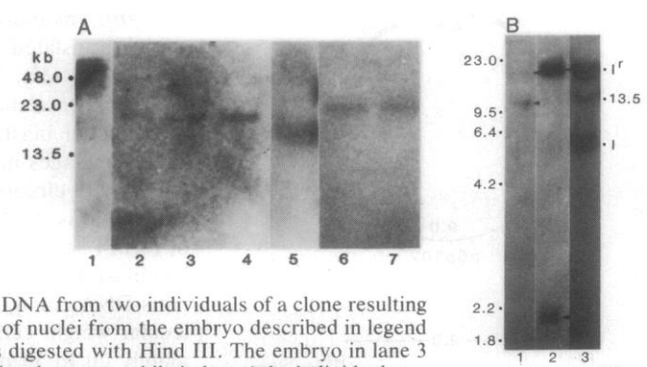

Fig. 3. (A) Hind III digests of clones of *Xenopus* embryos and larvae. (Lane 1) DNA from a single gastrula of a clone which was not treated with any restriction enzyme; (lane 2) DNA from a donor embryo at the gastrula stage treated with Hind III; (lanes 3 and 4) DNA from two individuals of a clone resulting from the transplantation of nuclei from the embryo described in legend of lane 2. The DNA was digested with Hind III. The embryo in lane 3 was at gastrula stage of development, while in lane 4 the individual was a stage 42 larva (*19*); (lane 5) Hind III digest of DNA from an individual neurula of a third clone; (lane 6) Hind III digest of DNA from a donor gastrula; (lane 7) DNA from an individual from a clone produced by transferring nuclei from the donor mentioned in lane 6 into an activated egg. The DNA was digested with Hind III. (B) Autoradiograph of putative junction fragments produced by Bam HI digestion of DNA from nuclear transplant animals. (Lane 1) Bam HI digested DNA from a single gastrula stage embryo of one of the clones described in (A); (lane 2) Bam HI digested DNA from a single larva from a different clone; (lane 3) pSp102 DNA mixed with *Xenopus* DNA.

DNA was analyzed from individuals of four clones (Fig. 3A). The pSp102 DNA sequences migrated with high molecular weight DNA in an individual neurula when the DNA was not digested (lane 1). Hind III digests of DNA from different individuals (including the donor and recipient) of a second clone showed similar restriction fragments, approximately 22 kb in size. This band differed from the Hind III digested DNA pattern of individuals from several other clones (Fig. 3A, lanes 5, 6, and 7). Bam HI digestion of DNA from individuals of different clones showed different patterns of putative junction fragments, suggesting that the genes are located in different chromosomal locations (Fig. 3B).

In some experiments we observed different patterns of Hind III generated restriction fragments within a single clone of embryos (data not shown). This suggested that some of the donor embryos were mosaics in which pSp102 was integrated into different sites in various cells. Clones of frogs with the gene located at the same position may be produced from mosaic embryos by serial nuclear transplantation.

Our results suggest that the original plasmid DNA was integrated into the DNA of the donor nuclei and can be transmitted to a clone of embryos by nuclear transplantation. We do recognize, however, that unequivocal proof of integration depends on the molecular cloning of putative junction fragments. Our procedure makes possible (i) the production of large numbers of embryos that have the injected gene integrated into the same chromosomal location, and

(ii) subsequent analysis of the developmental and tissue specific expression of an exogenous sequence in embryos containing the gene integrated at the same site.

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Isolation of Human C-Reactive Protein Complementary DNA and Localization of the Gene to Chromosome 1

Abstract. *With a synthetic oligonucleotide mixture as probe, complementary DNA clones of C-reactive protein were isolated from an adult human liver complementary DNA library. The clones ranged in size from 700 to 1100 base pairs and were identified by partial DNA sequence analysis. One complementary DNA clone was used as a probe for hybridization with human-rodent DNA's isolated from somatic cell hybrids and bound to nitrocellulose filters (Southern blot analysis) to assign the human C-reactive protein gene to chromosome 1.*

Originally C-reactive protein (CRP) was defined as a substance—observed in the plasma of patients with acute infections—that reacted with the C polysaccharide of *Streptococcus pneumoniae* (*1*). Human CRP is outstanding among the plasma proteins that increase in concentration after tissue injury or inflammation (the acute phase proteins). During the acute phase response, the serum concentration of CRP may increase 1000 times or more, exceeding by several orders of magnitude increases in the concentrations of the other acute phase proteins (*2*). Human CRP has been purified to homogeneity, its complete amino acid sequence has been determined (*3*), and considerable information on the biological effects of CRP in man and experimental animals has been obtained (*4*). The isolation of complementary DNA (cDNA) probes for the CRP sequence would permit analysis of the acute phase response at the molecular level and a detailed description of the structure, chromosomal location, and expression of the CRP gene.

A synthetic oligonucleotide mixture, comprising all 17-nucleotide DNA sequences that could code for the amino acid sequence of CRP between residues 141 and 146 (*3*), was generated by a solid-phase phosphotriester method, using a library of dimer anions (*5*). Residues 141 to 146 of the amino acid sequence of human CRP display minimal ambiguity in codon assignment and required synthesis of only eight oligonucleotide sequences (Fig. 1). The oligonucleotide mixture was labeled at the 5′ end with adenosine [γ-^{32}P]triphosphate, treated with T4 polynucleotide kinase (*6*), and used as a hybridization probe to screen approximately 25,000 human liver cDNA clones (*5*). Fifteen clones hybridized specifically to the oligonucleotide mixture. After colony purification, plasmid DNA was isolated from five of the positive clones by the cleared lysate method (*7*). The cDNA inserts derived from the purified plasmids by digestion with the restriction endonuclease Pst I ranged in size from 700 to 1100 base pairs. The 5′ and 3′ ends of the largest clone, pCRP1, were sequenced (*6*). The nucleotide and derived amino acid sequences of the 5′ end of this clone are shown (Fig. 1). These sequences at the 5′ end define residues 107 to 138 in human CRP (*3*), thus confirming pCRP1 as a CRP-specific cDNA clone. Restriction enzyme mapping and the 5′ DNA sequence established the relation between the clone pCRP1, the native protein, its messenger RNA (mRNA), and the synthetic oligonucleotide mixture (Fig. 1). The amino acid sequence of CRP comprises 187 residues (*3*), requiring an mRNA of at least 561 nucleotides, not including the 3′ and 5′ untranslated regions. Partial characterization of pCRP1 (Fig. 1) shows that this clone contains 243 nucleotides of the CRP coding sequence corresponding to the region from residue 107 to the carboxyl terminus and 600 nucleotides of the 3′ untranslated region.

The chromosomal location of a number of human genes has been established with the use of specific DNA clones as hybridization probes to identify human gene sequences in the Southern blot analysis of DNA samples isolated from

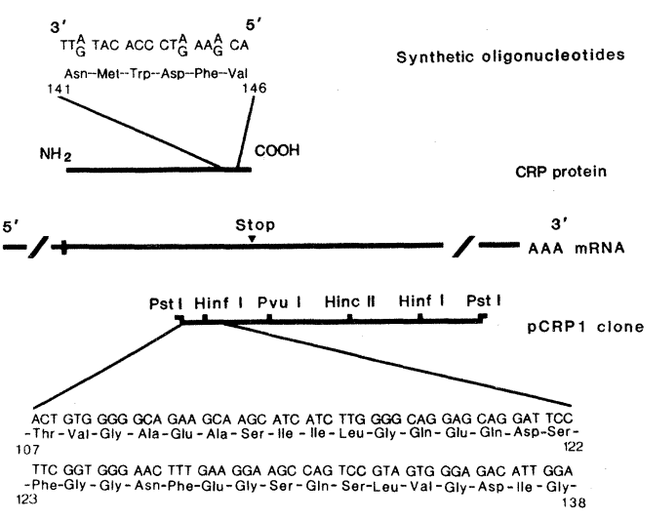

Fig. 1. Relation between the pCRP1 clone, the native C-reactive protein, its mRNA, and the synthetic oligonucleotide mixture. The 5′ nucleotide sequence and derived amino acid sequence establish the identity and position of the clone relative to the protein. Internal restriction endonuclease sites are Pvu I, Hinf I, and Hinc II.