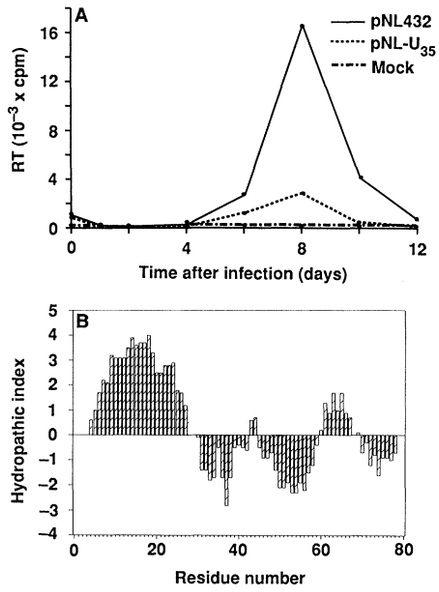

Fig. 3. (**A**) Infection of A3.01 cells with virus defective in *vpu*. SW480 cells, cultivated in DMEM + 10% fetal calf serum (FCS), were transfected with pNL-U_{35} DNA or with the wild-type plasmid DNA pNL432 using the standard calcium phosphate precipitation technique (*14*). After the transfection, SW480 cells were cultivated in RPMI 1640 containing 10% FCS. Supernatants were collected 20 hours after the transfection and filtered (0.22 μm). The RT activity of the supernatants was subsequently determined and equal RT-units, corresponding to 2.0 to 2.8 ml of the supernatant, were used to infect 2×10^6 A3.01 cells in a total volume of 5 ml of RPMI 1640. RT activity in the supernatants was determined at the indicated time points in a standard RT assay (*15*). The values given represent enzyme activity present in 1.5 μl of culture supernatant. (**B**) Hydrophobicity profile of the predicted amino acid sequence encoded by the HIV-1 *vpu* gene. The relative hydrophobicity of the amino acid residues is shown as a function of the amino acid position in the predicted *vpu* protein of our LAV isolate, HIVBru (*16*). Values were calculated by the method of Kyte and Doolittle (*17*).

duction indicate that the cells infected with the *vpu* mutant contain up to five times more *gag*, *pol*, and *env* proteins than those producing wild-type particles (7). This intracellular accumulation of viral proteins coupled with decreased virion synthesis suggests that *vpu* has a role in virus assembly or maturation.

As shown in Fig. 3B, the NH_2-terminus of *vpu* contains a 27–amino acid stretch of hydrophobic residues, which is characteristic of membrane-associated proteins. Adjacent to this hydrophobic domain is a run of 32 residues that includes 15 positively or negatively charged amino acids. This hydrophilic region of the HIV-1 *vpu* protein is highly conserved among several HIV isolates. The *vpu* protein thus possesses some of the structural features of the small, membrane-associated proteins present in ortho- and paramyxovirus infected cells. For example, the 15-kD membrane-bound M2 protein of influenza virus contains a charged cytoplasmic tail and is thought to be involved in virus assembly (*8*).

Although it is possible that the functional *vpu* protein is expressed from several exons in addition to the *vpu* ORF, this seems unlikely since its size (16 kD) in cells transfected with pNL-A1 was indistinguishable from that observed after in vitro translation. A consensus splice acceptor is located within the first translated exons of *tat* and *rev*, 85 nucleotides upstream from the initiation codon of the *vpu* gene (position 6012); this AUG codon is the first that precedes an ORF.

It is of interest that a number of the sequenced HIV-1 proviruses lack a functional *vpu* gene. The published sequence for HXB2, BH8, Mal, and Z6 proviral DNAs do not contain the initiator AUG for the *vpu* ORF (*9*); the cloned ARV-2 proviral DNA contains a 1-bp deletion within *vpu* leading to a frame shift and termination after amino acid 38 (*10*). Since a functional *vpu* gene is not absolutely essential for the production of HIV-1, each of these isolates would give rise to progeny virions with altered replicative potential. In fact, variations in replicative rates, levels of progeny virion production, and cytopathicity have been described for HIV-1 isolates obtained at different points in disease (*12*) which may be relevant for understanding viral persistence.

REFERENCES AND NOTES

1. F. Wong-Staal, P. K. Chanda, J. Ghrayeb, *AIDS Res. Human Retrovir.* **3**, 33 (1987).
2. P. Sonigo *et al.*, *Cell* **42**, 369 (1985).
3. A. Adachi *et al.*, *J. Virol.* **59**, 284 (1986).
4. D. A. Melton *et al.*, *Nucleic Acids Res.* **12**, 7035 (1984).
5. K. Strebel *et al.*, *Nature* **328**, 728 (1987).
6. K. Strebel, unpublished observations.
7. T. Klimkait, in preparation.
8. R. A. Lamb *et al.*, *Cell* **40**, 627 (1985).
9. L. Ratner *et al.*, *AIDS Res. Human Retrovir.* **3**, 57 (1987); L. Ratner *et al.*, *Nature* **313**, 277 (1985); M. Alizon *et al.*, *Cell* **46**, 63 (1986); A. Srinivasan *et al.*, *Gene* **52**, 71 (1987).
10. R. Sanchez-Pescador *et al.*, *Science* **227**, 484 (1985).
11. J. Sodroski *et al.*, *ibid.* **231**, 1549 (1986).
12. M. Tersmette *et al.*, *J. Virol.* **62**, 2026 (1988).
13. R. Gallo *et al.*, *Nature* **333**, 504 (1988).
14. F. L. Graham and A. van der Eb, *Virology* **52**, 456 (1973); C. M. Gorman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6777 (1982).
15. S. Goff, P. Traktman, D. Baltimore, *J. Virol.* **38**, 239 (1981); R. L. Willey *et al. J. Virol.* **62**, 139 (1988).
16. S. Wain-Hobson *et al.*, *Cell* **40**, 9 (1985).
17. J. Kyte and R. F. Doolittle, *J. Mol. Biol.* **157**, 105 (1982).

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Platelet-Derived Growth Factor A Chain Is Maternally Encoded in *Xenopus* Embryos

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Transcription of zygotic genes does not occur in early *Xenopus* embryos until the mid-blastula transition, 6 to 7 hours after fertilization. Before this time, development is directed by maternal proteins and messenger RNAs stored within the egg. Two different forms of the A chain of platelet-derived growth factor (PDGF) are shown here to be encoded by maternal messenger RNAs. The two forms closely resemble human PDGF; however, the long form contains a hydrophobic region near the carboxyl terminus. The presence of PDGF messenger RNA in the embryo supports the idea that endogenous growth factors act at the earliest stages of embryogenesis.

CONDITIONED CULTURE MEDIUM from murine embryonal carcinoma cells contains a PDGF-like activity (*1*). These cells resemble derivatives of the inner cell mass of the mouse blastocyst, thus PDGF may be involved in early mammalian development. Because of the difficulties in manipulating the mammalian embryo, we wished to determine if PDGF participates in the early development of the experimentally more accessible *Xenopus* embryo.

In humans, two peptide chains encoded by separate genes form three biologically active isoforms of PDGF, the homodimers A:A and B:B and the heterodimer A:B (*2*). We used human A chain cDNA (*3*) and simian sarcoma virus v-*sis* (B chain) clones (*4*) as hybridization probes for the DNA blot analysis of *Xenopus laevis* genomic

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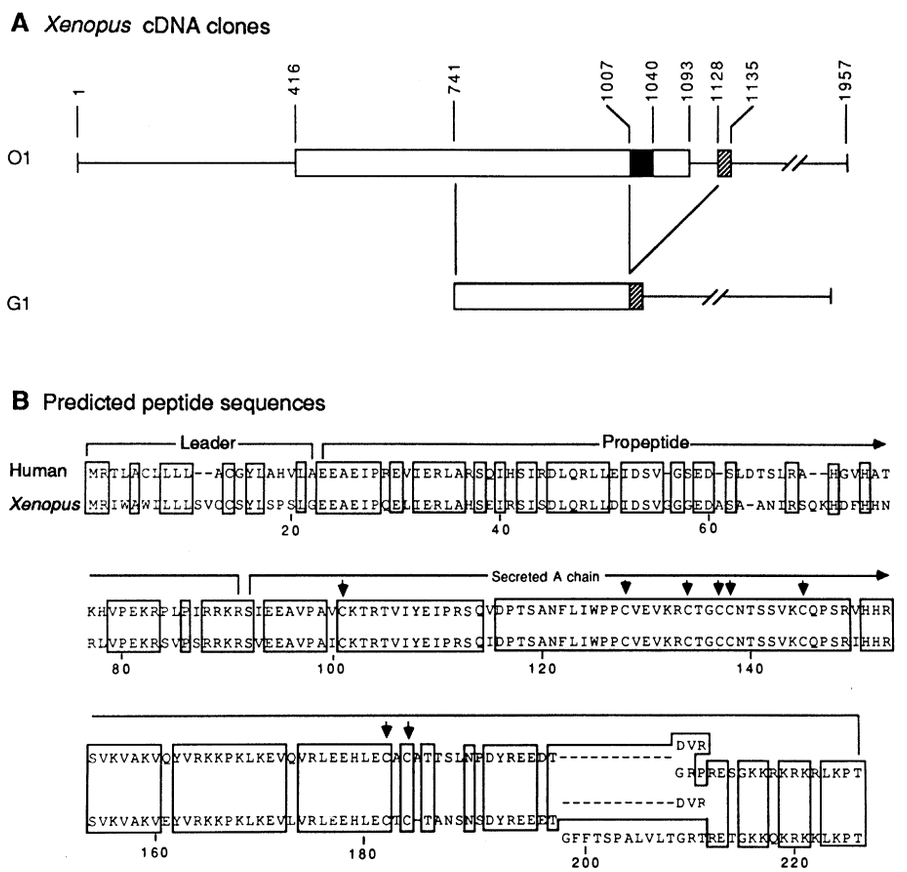

Fig. 1. *Xenopus* A chain cDNA clones. (**A**) Comparison of O1 and G1 cDNA clones. The coding regions are indicated by boxes. The insertion in O1 not contained in the human cDNA (*4*) is shown filled. The hatched region in O1 corresponds to the COOH-terminal region expressed in G1. The base pair numbers shown above refer to the sequence of O1 (sequence provided upon request). The relative position of G1 with respect to O1 is shown. (**B**) The predicted amino acid sequences of the long and short forms of the *Xenopus* and human A chain precursor peptides. The residue numbers refer to the *Xenopus* peptide. Regions of identity are boxed. Arrowheads indicate the eight conserved cysteine residues of the secreted peptide.

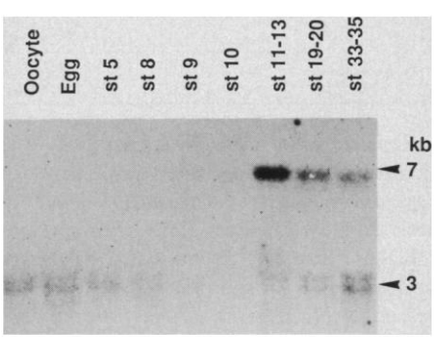

Fig. 2. RNA blot analysis of RNA from *Xenopus laevis* oocytes and early embryos. RNA from ten stage VI oocytes or staged (st) embryos was electrophoresed in 1% agarose-formaldehyde gels and transferred to nitrocellulose. Embryo stages are as in reference (*18*). An antisense transcript of the *Xenopus* A chain cDNA was synthesized in the presence of [^{32}P]CTP (cytidine 5′-triphosphate) with T7 polymerase as described (*19*) and used as a hybridization probe under stringent conditions (5× SSPE, 50% formamide, and 0.5% SDS at 60°C; the filter was washed in 0.1× SSC and 0.5% SDS at 65°C). The filter was exposed to Kodak XAR-5 film with an intensifying screen at −70°C for up to one week. Sizes correspond to molecular size standards.

DNA. The different probes detected different fragments (*5*), indicating that *Xenopus*, like humans, has distinct A and B chain genes.

Oocyte and gastrula stage cDNA libraries (*6*) were screened by hybridization to the A chain and B chain DNA fragments. Of the approximately 300,000 individual clones of each library screened, four clones from the oocyte library and one from the gastrula stage library hybridized to the A chain probe. No clones hybridized to the B chain probe.

Two different classes of cDNA were isolated (Fig. 1A). The longest oocyte clone, O1, encodes a protein resembling the long form of A chain, which was originally identified as the product of glioma cell lines (*3*). The gastrula stage clone, G1, is identical to O1 over its entire length except that the region between 1008 and 1127 has been deleted. G1 encodes the short form of A chain, which was originally isolated from endothelial cell lines, and is also present in glioma lines (*7*). Thus, as for humans, where the two forms appear to differ by the alternative splicing of a 69-bp exon (*7*), O1 and G1 appear to be the products of alternative splicing of a 121-bp region.

The sequence of O1 was compared to the human cDNA (*3*). The translation start site is assigned to nucleotide 416 in agreement with the position in the human cDNA. In both species, the sequence surrounding the initiation AUG (CGCAAUGA in *Xenopus* and CGCGAUGA in humans) weakly conforms to the optimal sequence for translation initiation (*8*). An in-frame termination codon at position 1094 also coincides with the human sequence. The coding region shows an overall sequence identity of 73%; the only major divergence is an insertion at the 3′ end which is depicted by a solid black region in Fig. 1A. Both the 5′ and 3′ untranslated regions are well conserved, having 53 and 65% sequence identity, respectively.

O1 and G1 encode peptides with different predicted COOH-termini. The predicted precursor protein products for both of these forms were compared to their human homologs (Fig. 1B). The O1 precursor protein contains 226 amino acids (~26 kD). As in the human A chain protein, the start methionine precedes a hydrophobic signal peptide region, which may be cleaved between Gly^{22} and Glu^{23}, conforming to the preferred leader peptidase cleavage sites (*9*). By analogy to the human homolog, a 69–amino acid propeptide may be cleaved to yield a 135–amino acid A chain peptide. After NH_2-terminal modification, the A chain protein would be approximately 15 kD and share 79% overall sequence identity with the predicted human A chain protein.

The predicted protein products of O1 and the human cDNA differ significantly only by an 11–amino acid insertion in the COOH-terminal region (black region in Fig. 1A), which introduces a 9–amino acid hydrophobic domain into the normally hydrophilic COOH-terminus (*10*). Whether this region is normally present in the growth factor or results from incomplete splicing of the mRNA is unclear. However, it does not appear to block mitogenic action of the peptide. Recombinant O1 and human peptides expressed in COS cells have comparable mitogenic activity for Balb/c 3T3 cells (*5*).

The isolation of oocyte and gastrula stage cDNA for PDGF A chain demonstrates that the corresponding mRNA is present during early development. We examined the pattern of expression during embryogenesis by RNA blot analysis with an antisense RNA transcript of the O1 cDNA as a hybridization probe (Fig. 2). Oocytes and cleavage stage embryos expressed at least two transcripts of ~3 kb that decreased in abundance during development. The transcripts reap-

pear at the late gastrula stage (stages 11 to 13) after the onset of embryonic transcription at the mid-blastula transition (stage 8) (*11*). At this time, however, a larger transcript of ~7 kb predominated. On longer exposures, the ~7-kb transcript was visible in RNA from oocytes and diminished in abundance in early cleavage stage embryos. The relation of the ~7-kb transcript to the smaller species is unknown. It may be an incompletely spliced form or precursor of the ~3-kb mRNA. The presence of multiple A chain transcripts in *Xenopus* oocytes and embryos is reminiscent of the expression of transcripts ranging from 1.9 kb to 3 kb in human cells (*3, 7*).

Although a *Xenopus* PDGF B chain gene was detected by DNA blotting with a v-*sis* probe, RNA blot analysis of RNA samples identical to those shown in Fig. 2 revealed no evidence of B chain mRNA (*5*). This result, combined with the lack of B chain cDNA clones in the oocyte and gastrula stage libraries, indicates that the B chain mRNA is present at a much lower level than the A chain mRNA within the early embryo, if at all.

Abundance of the A chain mRNA was estimated by its frequency of recovery from oocyte and gastrulation stage cDNA libraries. Each mature oocyte contained about 90 ng of polyadenylated mRNA corresponding to about 2×10^{10} mRNA molecules (*12*). Based on a representation of four copies per 300,000 clones in the cDNA library, a mature oocyte would contain about 5×10^5 A chain mRNA transcripts.

The isolation of the long form of A chain from an oocyte library and the short form from the gastrula library does not necessarily imply a developmental program of alternative splicing. Both forms are present in human cell lines expressing A chain (*7*) and in early mouse embryos (*5*). Furthermore, RNA protection data from experiments with a hybridization probe spanning the splice junction show that the short form predominates in both oocytes and stage 11 to 13 embryos (*5*). It is not yet known what, if any, functional differences distinguish the two forms.

Recently, two other growth factors have been implicated in the control of early *Xenopus* development. Early embryos contain a maternal mRNA containing sequences complementary to fibroblast growth factor (FGF) (*13*). *Vg1*, which encodes a molecule related to transforming growth factor–β (TGF-β), is also maternally encoded (*14*). Thus, growth factors may act during oogenesis or the first stages of embryogenesis. Growth factors may promote the rapid cell division seen in the early embryo, and purified FGF and TGF-β can mimic some aspects of the induction of mesoderm which normally occurs at gastrulation (*13, 15*). While these studies did not use proteins isolated from early *Xenopus* embryos, they suggest that endogenous growth factors may regulate differentiation. Recently, a paradigm for the control of differentiation by PDGF has come from studies of rat astrocytes (*16*). PDGF-like molecules have been shown to inhibit differentiation by promoting the division of a progenitor cell. It is conceivable, therefore, that PDGF A chain controls differentiation in *Xenopus* embryos by a similar mechanism. The experimental accessibility of the *Xenopus* embryo in conjunction with recent advances in antisense nucleotide technology will aid in determining the role of PDGF in development.

Note added in proof: The PDGF A chain mRNA has been observed in mouse eggs and preimplantation embryos (*17*).

REFERENCES AND NOTES

1. L. J. Gudas, J. P. Singh, C. D. Stiles, *Cold Spring Harbor Conf. Cell Proliferation* **10**, 229 (1983); A. Rizzino and D. F. Bowen-Pope, *Dev. Biol.* **110**, 15 (1985).
2. R. Ross *et al.*, *Cell* **46**, 155 (1986).
3. C. Betsholtz *et al.*, *Nature* **320**, 697 (1986).
4. S. G. Devare *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 731 (1983).
5. M. Mercola and C. D. Stiles, unpublished results.
6. M. R. Rebagliati *et al.*, *Cell* **42**, 769 (1985); P. A. Krieg and D. A. Melton, *EMBO J.* **4**, 3463 (1985).
7. B. D. Tong *et al.*, *Nature* **328**, 619 (1987); T. Collins, D. T. Bonthron, S. H. Orkin, *ibid.*, p. 621; D. T. Bonthron, C. C. Morton, S. H. Orkin, T. Collins, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1492 (1988).
8. M. Kozak, *Cell* **44**, 283 (1986).
9. G. J. Von Heijne, *J. Mol. Biol.* **173**, 243 (1984).
10. Hydropathicity determined by the method of J. Kyte and R. F. Doolittle, *J. Mol. Biol.* **157**, 105 (1982).
11. J. Newport and M. Kirschner, *Cell* **30**, 675 (1982).
12. E. Davidson, *Gene Activity in Early Development* (Academic Press, Orlando, FL, 1986), pp. 366–369.
13. J. M. W. Slack *et al.*, *Nature* **326**, 197 (1987); D. Kimmelman and M. Kirschner, *Cell* **51**, 869 (1987).
14. D. L. Weeks and D. A. Melton, *Cell* **51**, 861 (1987).
15. S. F. Godsave *et al.*, *Development* **102**, 555 (1988).
16. M. Noble, K. Murray, P. Stroobant, M. D. Waterfield, P. Riddle, *Nature* **333**, 560 (1988).
17. D. A. Rappolee, C. A. Brenner, R. Schultz, D. Mark, Z. Werb, *Science*, in press.
18. P. D. Nieuwkoop and J. Faber, *Normal Table of* Xenopus laevis (*Daudin*) (North-Holland, Amsterdam, 1975).
19. D. A. Melton *et al.*, *Nucleic Acids Res.* **12**, 7035 (1984).

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A Mutation of the Circadian System in Golden Hamsters

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A mutation has been found that dramatically shortens the period of the circadian locomotor rhythm of golden hamsters. The pattern of inheritance of this mutation suggests that it occurred at a single, autosomal locus (*tau*). Wild-type animals have rhythms with free-running periods averaging about 24 hours; animals heterozygous for the mutation have periods of about 22 hours, whereas homozygous animals have rhythms with periods close to 20 hours. Animals that carry the mutant alleles exhibit abnormal entrainment to 24-hour light:dark cycles or are unable to entrain.

GENETIC MUTATIONS THAT AFFECT the period (τ) of circadian rhythms have been studied and characterized extensively in nonvertebrate organisms and have provided a useful approach to the study of the molecular and biochemical mechanisms that generate and control rhythmicity. "Clock" mutants have been described in *Drosophila, Neurospora*, and *Chlamydomonas* (*1, 2*). However, single gene mutations that affect τ have not been described for any vertebrate.

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We found a single male hamster (*Mesocricetus auratus*; Charles River Breeding Labs) that exhibited an abnormally short τ in constant dark (DD). Whereas the normal τ_{DD} for golden hamsters averages about 24.1 hours and is rarely shorter than 23.5 hours (*3, 4*), the free-running period of the abnormal male was 22.0 hours and was stable for 3 weeks. When it was exposed to a light:dark cycle (LD) that consisted of 14 hours of light (100 lux) and 10 hours of dark (LD 14:10), the animal entrained. However, entrainment was abnormal; activity onsets occurred about 4 hours earlier than those of normal animals. We bred this animal with three female hamsters with normal freerunning periods (τ = 24.01, 24.03,