We propose that *sna* allows *twi* to function as a mesodermal determinant by repressing the expression of *sim* (*16*), AS-C (*10*), E(spl) (*25*), and other regulatory genes responsible for the differentiation of the mesectoderm and neuroectoderm. In *sna* embryos, ventral cells that normally form mesoderm now express the wrong regulatory genes, and consequently follow an alternative fate.

Note added in proof: Similar results on the role of *sna* in mesoderm formation have been obtained (*25a*).

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2. A synthetic 36-nucleotide oligomer was synthesized that is complementary to a region of the *sna* protein coding sequence (*1*). The oligomer was used to screen a 4- to 8-hour cDNA plasmid library (*26*). Three hybridizing clones were purified and their restriction maps were found to correspond to the *sna* map (*1*). One of the clones was partially sequenced so that its identity as a *sna* cDNA could be confirmed. An 880–base pair (bp) Nde I restriction fragment from the cDNA, containing coding sequences from amino acid residues 103 to 390 was inserted into the unique Nde I site of the bacterial pAR3040 T7 expression plasmid. The *sna* peptide was overexpressed in the BL21 (DE3) strain (*27*).
3. Preparative quantities of the *sna* peptide were isolated from SDS-polyacrylamide gels and used for immunization of a guinea pig (Pocono Farms, PA). The serum was diluted 1:200 in phosphate-buffered saline, 1% bovine serum albumin, 0.5 M NaCl, 0.1% Tween-80, and was used to stain whole-mount preparations of formaldehyde-methanol–fixed embryos (*28*). The protein was visualized using a tetramethyl rhodamine isothiocyanate-conjugated antibody to guinea pig (Jackson Immunoresearch, Bethesda, MD) diluted in the same staining buffer. Staining of *twi* was done with a rabbit antibody provided by S. Roth *et al.* (*29*), and visualized with a fluorescein isothiocyanate–conjugated secondary antibody (Jackson Immunoresearch). Fluorescence microscopy was done with a Nikon Optiphot microscope, and photographs were taken with Kodak Kodachrome film (Asa 64).
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30. RNAs were localized by means of the whole-mount in situ hybridization method developed by D. Tautz and C. Pfeifle [*Chromosoma* **98**, 81 (1989)]; the *sna* probe that was used for hybridization corresponded to an 880-bp Nde I restriction fragment from the full-length cDNA, whereas the *T3* probe was a 1-kb genomic DNA fragment (provided by J. Modellel and M. Caudy).
31. The following mutant alleles were used in this study: *twist*, ID96 (*29*); *snail*, IIG05 (*6*); and *dorsal*, dl-8 (*29*).
32. The *rho* probe used for whole-mount hybridizations corresponded to a 2.5-kb Eco RI restriction fragment from a cDNA (provided by E. Bier).

33. We thank M. Caudy and J. Modolell for *T3* DNA, E. Bier for the *rho* DNA, N. Brown for the cDNA library, S. Roth for the *twi* antibody, R. Kraut for helpful discussions, S. Small for help with the photography, and C. Rushlow, R. Warrior, B. Harris, and E. Bier for critical readings of the manuscript. Supported in part by the American Chemical Society and by NIH grant GM 46638.

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Technical Comment

Mathematical Analysis of a Model of the Mitotic Clock

In their mathematical model of the cyclin and maturation promotion factor (MPF) system, Norel and Agur (*1*) note that oscillations occurred if the degradation of cyclin had saturable kinetics but not if the reaction was first order with respect to cyclin. One might ask whether this saturability is mathematically necessary for oscillation, or whether the investigators just did not find the right parameters for the first-order case. In the same vein, one might ask whether the degradation of MPF must have saturable kinetics (as assumed) and whether the autocatalytic term for the formation of MPF must be of order greater than 1 with respect to MPF.

These questions can be answered by analyzing the effect of reaction order on stability, following Higgins (*2*). Let M and C denote the concentrations of active MPF and cyclin, respectively, and $\dot{M}$ and $\dot{C}$ the rates of change in these concentrations. For a closed trajectory around a single critical point [that is, a point (M,C) where $\dot{M} = \dot{C} = 0$], the Poincaré theorem requires

$$(\partial\dot{M}/\partial M)(\partial\dot{C}/\partial C) - (\partial\dot{M}/\partial C)(\partial\dot{C}/\partial M) > 0 \quad (1)$$

at the critical point. A sufficient condition for instability at the critical point is

$$\partial\dot{M}/\partial M + \partial\dot{C}/\partial C > 0 \quad (2)$$

These equations can be restated in terms of orders and rates of reaction at the critical point, the order of reaction with respect to X being defined as $(X/V)(\partial V/\partial X)$, where V is the reaction rate. So defined, reaction order may depend on concentration and must be evaluated at the critical point for our present purposes. The rates of change of M and C in the Norel and Agur model are the differences between rates of formation and rates of removal:

$$\dot{M} = F_M - R_M \text{ and } \dot{C} = F_C - R_C$$

Then, for example, $\partial\dot{M}/\partial M = \partial F_M/\partial M - \partial R_M/\partial M$. Let ϕ_{MX} and ρ_{MX} be the orders of reaction for F_M and R_M, respectively, with respect to X, for example,

$$\phi_{MM} = (M/F_M)(\partial F_M/\partial M)$$

Then, because $F_M = R_M$ and $F_C = R_C$ at the critical point,

$$\partial M/\partial M = (F_M/M)(\phi_{MM} - \rho_{MM})$$

and Eqs. 1 and 2 become

$$(\phi_{MM} - \rho_{MM})(\phi_{CC} - \rho_{CC}) - (\phi_{MC} - \rho_{MC})(\phi_{CM} - \rho_{CM}) > 0 \quad (3)$$

and

$$\frac{F_M}{M}(\phi_{MM} - \rho_{MM}) + \frac{F_C}{C}(\phi_{CC} - \rho_{CC}) > 0 \quad (4)$$

In the Norel and Agur model, $\rho_{MC} = \phi_{CM} = \phi_{CC} = 0$, and $\phi_{MC} = \rho_{CM} = 1$; so Eqs. 3 and 4 become

$$(\phi_{MM} - \rho_{MM})\rho_{CC} < 1 \quad (5)$$

and

$$(\phi_{MM} - \rho_{MM}) > \frac{MF_C}{F_M C}\rho_{CC} \quad (6)$$

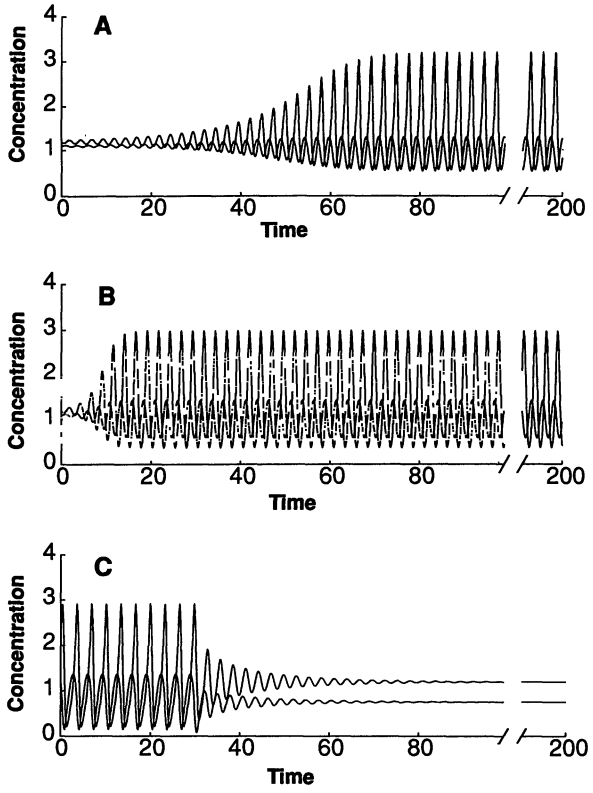

Fig. 1. Modifications of the model of Norel and Agur. The curves show changes in M and C concentrations over time (in all cases M has the larger oscillations) [see (*1*) for units]. (**A**) First order removal of M, removal of C first order with respect to C. Differential equations: $\dot{M} = (e + fM^2)C - gM$, $\dot{C} = i - MC$; parameter values $e = 2.88$, $f = 3.0$, $g = 6.6$, $i = 1.32$; critical point $M = 1.2$, $C = 1.1$; initial state $M = 1.19$, $C = 1.09$. (**B**) First-order autocatalysis in the formation of M, saturable removal of M, and first-order (with respect to C) removal of C. Differential equations: $\dot{M} = (e + fM)C - gM/(M + 1)$, $\dot{C} = i - MC$; parameter values $e = 2.4$, $f = 4.8$, $g = 16.456$, $i = 1.32$; critical point $M = 1.2$, $C = 1.1$; initial state $M = 1.19$, $C = 1.09$. (**C**) Stabilization of the critical point of the original Norel and Agur model by a competitive antagonist of M removal. Differential equations (*1*): $\dot{M} = (e + fM^2)C - gM/(M + h)$, $\dot{C} = i - MC$; parameter values $e = 3.5$, $f = 1.0$, $g = 10.0$, $i = 1.2$. For time $t < 30$ time units, $h = 1$ (no competitive antagonist); for $t \geq 30$, $h = 2$ (competitive antagonist present at a concentration equal to its dissociation constant). Initial state (a point on the limit cycle of the original model) $M = 2.10004647$, $C = 1.16628822$.

To satisfy Eq. 6, ϕ_{MM} must be greater than ρ_{MM} (assuming $\rho_{CC} \geq 0$); but neither Eq. 5 nor Eq. 6 bars first-order removal of C ($\rho_{CC} = 1$) or M ($\rho_{MM} = 1$); nor is it necessary that the autocatalytic order ϕ_{MM} exceed 1, provided $\rho_{MM} < \phi_{MM}$.

Finding a set of parameters to demonstrate oscillation is straightforward. Take the case where both removal reactions are first order, that is $R_M = gM$, $R_C = MC$, and $\rho_{MM} = \rho_{CC} = 1$. Because $\phi_{MM} < 2$ in the Norel and Agur model, Eq. 5 is satisfied. Equation 6 becomes

$$2\left[\frac{M^2}{e/f + M^2}\right] - 1 > \frac{M}{g} \qquad (7)$$

To match approximately the values of the Norel and Agur model, set $M = 1.2$ and $C = 1.1$ at the critical point; then to satisfy Eq. 7 let $M^2/[(e/f) + M^2] = 0.6$, making $e/f = 0.96$. In the steady state, $F_M = R_M$, that is $(e + fM^2)C = gM$; therefore

$$f = gM/\{[(e/f) + M^2]C\}$$

Let $g = 6.6$; then $f = 3.0$ and $e = 0.96f = 2.88$. Finally, $F_C = R_C$, that is $i = MC = 1.32$. Oscillations with these parameter values are shown in Fig. 1A (*3*).

In the same way, parameter values can be found for the case where the autocatalytic term is at most first order with respect to M, that is, $F_M = (e + fM)C$. In this case, R_M must be less than first order with respect to M so as to satisfy $\rho_{MM} < \phi_{MM}$ (Eq. 6). The case where $R_M = gM/(M + 1)$ and $R_C = MC$ is shown in Fig. 1B.

Equations 3 and 4 might be used to suggest what external interventions could be effective in altering stability. For the model of figure 1A of Norel and Agur (*1*), $R_C = M$ and $\rho_{CC} = 0$, and therefore Eq. 6 becomes

$$(\phi_{MM} - \rho_{MM}) > 0 \qquad (8)$$

This might be invalidated either by decreasing ϕ_{MM} or by increasing ρ_{MM}. Because

$$\phi_{MM} = 2M^2/[(e/f) + M^2]$$

ϕ_{MM} can be decreased by an agent that either decreases the ratio f/e or decreases M (which, in this model, can only be done by decreasing i). Alternatively, ρ_{MM} can be increased (to a maximum of 1) by a competitive antagonist of M removal; and, if $\phi_{MM} < 1$, then Eq. 8 can be invalidated and instability abolished. With a competitive antagonist, $R_M = gM/(M + h)$ and $\rho_{MM} = h/(M + h)$, where $h = 1$ in the absence of the antagonist and is increased by addition of the antagonist (*4*) (Fig. 1C).

These remarks concern only the stability at the critical point and do not challenge the conclusions of Norel and Agur on the main question of the modulation of cell cycle duration.

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