Prokaryotic and eukaryotic cell-cycle proteins

SIR—We report here what we believe to be the first demonstration of homology between prokaryotic and eukaryotic cell-cycle proteins. It is the result of a comparison of the predicted amino-acid sequence of the *Escherichia coli* cell-cycle protein FtsA[1] determined by Robinson *et al.*[2] with the National Biomedical Research Foundation protein sequence database (Version 8; 809,386 residues) using the 'best local similarity' algorithm of Smith and Waterman[3] as implemented on an ICL DAP supercomputer by Lyall *et al.*[4]. A search of this type can give valuable insights into the possible functions and origins of predicted gene products as similarity of sequence suggests similarity of structure and function.

Alignments of amino-acid sequence are selected by the program from all those possible, including insertions and deletions where required in order to improve the quality of the alignment. Insertions and deletions are penalized to be worse than the worst mismatch scored. The analysis revealed a highly significant similarity, extending over a region of 60 amino acids, between the primary sequence of FtsA and the cell-cycle proteins CDC28 from the budding yeast *Saccharomyces cerevisiae*[5] and CDC2 from the fission yeast *Schizosaccharomyces pombe*[6]. The figure shows the extent of similarity observed between the three proteins.

We used the conserved elements in the region shown as a pattern to re-search the database for related segments and to assess their significance. The alignment of the conserved pattern with CDC28, for example, scored more than 60 standard deviations above the expected frequency, based on statistical analysis of the distribution of the scores of the best alignments. The homology reported between these proteins is therefore extremely significant; the next best alignment scored only 2 standard deviations above expectation, and is not significant. There is no significant homology to human CDC2Hs[7].

What might be the reason for this striking similarity between *E. coli* and yeast cell-cycle proteins? The functions of these proteins (other than the kinase activities observed *in vitro* for CDC28 and CDC2[8,9]), or their modes of action at the molecular level, are not known, but their similarity in this 60 amino-acid region suggests a common property. There is no direct evidence that FtsA shares the kinase function of the yeast proteins as the similarity found does not extend to the flanking regions in CDC28 and CDC2 that are identical to consensus sequences for ATP binding and phosphorylation sites. However, a nucleotide-binding motif[10] (G----GK, residues 381–387) is found in FtsA distal to the region of similarity and a possible phosphorylation site (the dipeptide YT, residues 221–222) is proximal to the region. It is possible that this represents a reversal of the domain order between FtsA and the yeast protein kinases. Such domain order reversals have been postulated to occur in a ribosomal protein[11].

We may be dealing with a case of convergent evolution, as *E. coli*, *S. cerevisiae* and *S. pombe* are genetically highly diverse. Another possibility, is that this 60 amino-acid region arose in a common ancestor before the emergence of the eukaryotes. In that case changes in the domain's amino-acid sequence throughout the course of evolution will have been severely constrained by a constant functional requirement.

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FtsA    L N L V N E E I L Q L Q E K L R - Q Q G V - K H H - L A A G
CDC28   L Y L V F E - F L D L D L K - R Y M E G I P K D Q P L G A D
CDC2    L Y L V F E - F L D M D L K - K Y M D R I S E T G A T S L D

FtsA    - - I V L T G G A R Q I - E G L A A C - A Q R V F H T Q V R
CDC28   - - I V - K K F M M Q L C K G I A Y C H S H R I L H R D L K
CDC2    P R L V - Q K F T Y Q L V N G V N F C H S R R I I H R D L K
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Comparison of amino-acid sequences from segments of the FtsA protein of *E. coli*[2] (residues 305–357), the CDC28 protein of *S. cerevisiae*[5] (residues 84–138) and the CDC2 protein of *S. pombe*[6] (residues 80–136). The sequences are aligned for maximum homology[4]. Amino-acids which are identical in FtsA and one or both of CDC28 and CDC2 are shown in solid-lined boxes, with conservative amino-acid changes in dashed-lined boxes. Related amino acids are grouped as follows: P,A,G,S,T — neutral, weakly hydrophobic; Q,N,E,D — hydrophilic acid amine; H,K,R — hydrophilic, basic; L,I,V,M — hydrophobic; F,Y,W — hydrophobic, aromatic.

Fertilization events

SIR—Dale[1] misrepresents the state of knowledge on the mechanism of fertilization. The existence of a rapid electrical block to polyspermy is solidly supported by data from sea urchins, starfish, the marine worm *Urechis* and frogs, where it has been shown that a rapid positive-going shift in the egg membrane potential occurs in response to insemination, that positive membrane potentials during insemination inhibit sperm penetration, and that negative potentials promote polyspermy[2,3]. The recent experiments[4–7] cited by Dale do not cast doubt on this hypothesis.

In fact, Shen and Steinhardt[4] confirmed that positive potential inhibits sperm entry and negative potentials promote it: when periods of positive potential were interrupted by transient (10–80 ms) shifts to negative potentials, the more negative the potential, the more probable was fertilization. Although 2–3 dozen sperm were bound to an egg at the time the negative voltage windows were applied, only 43 out of 145 eggs were fertilized and most of these were monospermic. This does not contradict the existence of an electrical polyspermy block, because the more negative the voltage and the longer the duration of the voltage window, the more eggs were fertilized, as would be predicted.

Furthermore, the incidence of polyspermy during the negative voltage windows was not less than expected. Eighteen of the 43 eggs that were fertilized during the voltage windows were scored for polyspermy: one was dispermic and the rest monospermic. When the data are analysed by the Poisson distribution an expected frequency of 3 per cent is calculated for dispermic eggs close to the observed frequency of about 2 per cent.

Dale himself states of his data[5] "The present report does not directly address the question of a fast block". In the other experiments[6,7], he cites sperm concentrations were deliberately kept low to avoid the polyspermy that would otherwise occur in eggs voltage-clamped to −20 mV. Thus there are no recent data that cast doubt on the occurrence of an electrical polyspermy block. To avoid confusion it should be pointed out that while some species possess electrical polyspermy blocks, others do not. Exceptions include hamster, the fish *Oryzias*, and salamanders (see ref. 2).

The other question raised by Dale was how sperm initiate egg activation. He suggested that if fusion and the first event in egg activation are simultaneous then it is more likely that the sperm injects materials into the egg through the cytoplasmic bridge formed at sperm–egg fusion than that there is the type of interaction in which sperm act as a ligand and the egg as a receptor. This is not logical. If fusion and the initiation of activation occur at the

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same time, the most that can be concluded is that fusion could cause activation, but it is no less likely that there is another cause. In fact, we have shown in *Urechis* that protein isolated from sperm acrosomal granules causes activation of eggs, including the electrical response[8,9]. Thus activation does not require sperm–egg fusion.

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AIDS predictions

SIR—In criticizing our work, May and Anderson[1] not only refer to its presentation at the WHO meeting on the containment of AIDS (acquired immune deficiency syndrome) in Geneva when they mean the WHO meeting in Graz[2] but they also mistakenly characterize our work[2-4] as an exercise in curve fitting. There is no joint work by us based on statistical analyses to fit polynomial or exponential curves to existing data on the incidence of AIDS. On the contrary, like May and Anderson[1], we have used the approximation of exponential growth for the number of human immunodeficiency virus (HIV) carriers to describe the initial stage of the epidemic. Taking into account the incubation time distribution we have studied the resulting incidence of AIDS. We have shown that the long and variable incubation times lead to transient phenomena characterized by an increase of the doubling times in the observable AIDS epidemic, of the incubation times, of the ratio of AIDS cases to HIV carriers, and so forth. As a consequence, the incidence of AIDS cases in the initial stage of the epidemic is nonexponential, notwithstanding the assumed exponential spread of the virus. In all our work we have stressed that the early exponential phase of growth of HIV carriers passes because of the depletion of the susceptible population and other inhibitory factors. We have looked at this depletion to provide upper limits for the duration of the exponential phase in various countries.

May and Anderson have a point in that extrapolation of trends gained by curve-fitting is unsafe, specially if done uncritically over an extended period. One of us (M.G.K.) made precisely this point in Anderson's presence at the Bilthoven meeting, December 1986. There are many statistical analyses of AIDS based on curve fitting procedures with extrapolation over several years, some of them made at influencial institutions (at the US Centers for Disease Control for example). Why, then, do May and Anderson aim their critical remarks only at our joint work, which has nothing to do with curve-fitting, and a very cautious projection for the United Kingdom[5] made at a time when other prognostic analyses were scarce?

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Screwworm eradication and climate

SIR—I am surprised that Krafsur and his newly acquired transatlantic colleagues[1] can dismiss my analysis[2] of the screwworm data so lightly. In respect of my equations (3) and (4), predicting seasonal numbers of screwworm cases in Texas from winter temperatures and summer temperatures, the regression coefficients for temperature are significant at the $P<0.01$ and $P<0.02$ levels, respectively, and crucially affect the model's predictability (see Fig. 4)[2]. How can they dismiss my conclusions as "an artefact of pooling heterogeneous data", especially when they agree that "exceptional weather can have dramatic effects on screwworm incidence"?

In fact, if my model is applied to their new data for the seven separate climatological divisions in Texas, assuming that autumn cases (A) represent on average half the preceding summer's cases (the actual proportion does not matter), then the number of winter cases (W) in any particular region can be predicted from winter temperature by equation (1) in Table 1. The parameters are clearly significant (see box), and, in fact, winter temperature looms larger in importance than autumn cases in the prediction (R^2 = 0.49, and 0.44, respectively). In the winter o summer model, equation (2), summer temperature is just short of being significant ($P>0.05$), but that is not surprising in such a small data set involving highly mobile fly populations.

Moreover, since publishing my analysis I have stumbled across independent evidence in support of the idea that change in climate rather than the release of sterile males might be responsible for screwworm eradication.

In their study of case incidence in various counties of South Texas in 1975–76, Krafsur and Garcia[3] inadvertently provide a fix on the overwintering temperature threshold for screwworm. In terms of reported cases, overwintering was possible only in the western counties of Webb, Zapata, J. Hogg, Star and Hildago where winter temperatures are about 2.7°C warmer than the average for South Texas as a whole (see graphs and p.691 in ref. 3). The average winter temperature for South Texas in 1975–76 was in fact 14.4°C (see Fig. 2 of ref. 3) so that an overwintering threshold of about 17.0°C is indicated.

This implies that the species would have been unable to overwinter even in southernmost Florida in 1957–58 when the mean winter temperature at Miami, for example, was only 16.7°C, the coldest on record in nearly 100 years. Similarly, in more recent times, the species could not have overwintered at, for example, Brownsville in southern Texas in 1977–78 or 1978–79, when the outbreaks collapsed and when the winters were the second and third coldest on record, nor in the winter of 1976–77 or any of the winters from 1982 to 1985, which were also very cold. More significantly, in relation to the current campaign in Mexico, the species would have experienced difficulty

	estimate	±	s.e.m.	t	P
a (intercept)	−22.94		3.66	6.26	<0.01
b (autumn cases)	1.49		0.34	4.40	<0.01
c (winter temperature)	0.28		0.05	5.40	<0.01

Table 1 Total reported cases of screwworm and average temperature for seven regions in Texas over 22 years[1]

Region	Screwworm cases			Temperature (°F)	
	Autumn* (A_{n-1})	Winter (W_n)	Summer (S_n)	Winter (wt_n)	Summer (st_n)
Low Plains	2,010	2	4,020	43.2	81.4
Trans Pecos	4,635	11	9,270	46.8	80.1
East Texas	1,434	0	2,868	47.6	80.9
Edwards Plateau	11,475	137	22,951	47.8	81.3
South Central	8,631	103	17,263	53.4	82.9
Southern	9,633	830	19,265	55.4	84.6
Lower Valley	1,670	194	3,340	59.7	83.9

*Autumn cases assumed to be ½ summer cases.

$$\ln(W_n+1) = -22.94 + 1.49\ln(A_{n-1}+1) + 0.28\,wt_n, \quad R^2 = 0.93, P<0.001. \qquad (1)$$

$$\ln(S_n+1) = 41.16 + 0.45\ln(W_n+1) - 0.41\,st_n, \quad R^2 = 0.68, P<0.05, st_n \text{ not significant}, P>0.05. \qquad (2)$$

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