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 FtsA¹ 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 Waterman³ 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 CDC2Hs⁷.

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 motif¹⁰ (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 protein¹¹.

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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1. Donachie, W.D. & Robinson, A.C. in *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology (ed. Neidhart, F.C.) 1578–1593 (American Society for Microbiology, Washington, DC, 1987).
2. Robinson, A.C. *et al.* *J. Bact.* **160**, 546–555 (1984).
3. Smith, T.F. & Waterman, M.S. *J. molec. Biol.* **147**, 195–197 (1981).
4. Lyall, A., Hill, C., Collins, J.F. & Coulson, A.F.W. in *Parallel Computing '85* (eds Feilmeier, M., Joubert, G. & Schendel, U.) 235–240 (North Holland, Amsterdam, 1986).
5. Lörincz, A.T. & Reed, S.I. *Nature* **307**, 183–185 (1984).
6. Hindley, J. & Phear, G.A. *Gene* **31**, 129–134 (1984).
7. Lee, M.G. & Nurse, P. *Nature* **327**, 31–35 (1987).
8. Reed, S.I., Hadwiger, J.A. & Lörincz, A.T. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4055–4059 (1985).
9. Simanis, V. & Nurse, P. *Cell* **45**, 261–268 (1986).
10. Walker, J.E., Sarste, M., Runswick, M.J. & Gay, N.J. *EMBO J.* **1**, 945–951 (1982).
11. Liljas, A. & Thirup, S. *Chemica Scripta* **26B**, 109–119 (1986).

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 T S E T G A T S L D
FtsA	- - I V L T G G A R Q T - E G L A A C - A Q R V F H T Q V R
CDC28	- - I V - K K F M M O L C K G I A Y C H S H R I L H R D L K I
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 I

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 homology³. 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—Dale¹ 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 Steinhardt⁴ 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 data⁵ "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