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Triggering positive competition

Ada Yonath

has to remain longer *in vitro*. Perturbations of genomic imprinting due to the possibly longer time needed in culture are the underlying scientific concern. In imprinting, the expression of certain genes depends on whether they come from the mother or father; culture media may or may not mimic the milieu of the human reproductive tract in directing correct parental expression.

Despite these reservations, the derivation of hES cell lines from a single human blastomere using clinical PGD is realistic, and offers an attractive way out of an ethical conundrum. As Irving Weissman remarked in a previous News & Views article[7], some politicians are already advocating that generating hES cells from the ICM should cease. Research would be redirected towards blastomere-derived or 'inhibited' embryonic lines (Box 1) — if indeed stem cells of embryonic origin are not abandoned altogether in favour of cell lines solely of adult origin, should that more demanding approach prove feasible. Yet efficacy of the alternatives to ICM-derived lines remains unclear. The reality is that ICM-derived hES cell lines exist, and in increasing number. The same cannot be said about the alternatives: research with ICM-derived hES cells should not be held in abeyance.

In the long term, will the method of deriving hES cells matter? Perhaps not. If we achieve a successful example of human stem-cell therapy with ICM-derived cells, the controversy over the provenance of the cells is likely to dissipate in large part. After all, opprobrium was initially heaped on advocates of prenatal genetic diagnosis in the 1960s, of *in vitro* fertilization in the 1970s, and of PGD in the 1990s. Each success was followed by widespread acceptance of these procedures[8]. Time and public opinion move on. ■

Box 1 | Other prospects for producing stem cells

● **'Inhibited' cell lines.** This was suggested by work[9] in which a gene inhibiting expression of another gene, *cdx2*, was inserted into an embryonic donor cell; *cdx2* is required for establishing the trophectoderm necessary for placental formation. After pluripotent stem cells developed, the inhibiting gene was removed. This allowed differentiation into a stem-cell line, but too late for potential implantation. The approach should mollify those fearful of reproductive cloning, but it does not address the fundamental concern over disturbing the inner cell mass.

● **Stem cells from adults.** Although this would be ideal, efficacy remains highly speculative.

● **Targeted, patient-specific hES cells.** This process is even more challenging, and requires a process called somatic cell nuclear transfer. The nucleus of an immature egg, an oocyte, is removed and in its place is inserted a nucleus of an adult cell from the patient in question. The ES cell line generated will thus have the patient's genotype, and immunological incompatibility should not be a problem.

As discussed in the main text, however, the derivation of hES cells from the inner cell mass (Fig. 1c) remains the most successful route. **J.L.S.**

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In most bacteria, a molecule known as trigger factor prevents misfolding of newly made proteins emerging from their ribosome factory. The dynamic action of this molecule has been followed using fluorescence spectroscopy.

Proteins have specific structures designed for their tasks, the fold of each protein being dictated by its amino-acid sequence. Within the cell, protein folding occurs as the protein is being made by a multi-subunit complex called the ribosome. Before the protein is long enough to acquire its final fold, one end of it emerges from the protective exit tunnel of the ribosome into the crowded cellular environment. To promote efficient folding under these unfavourable conditions, all cells contain molecular 'chaperone' proteins. The various effects exerted by these chaperones are only partly understood, but some of them have the task of preventing the aggregation and misfolding of the emerging proteins[1], mostly by transiently masking 'sticky' hydrophobic surfaces. These sticky patches will generally become buried inside the mature protein, but may be exposed on the elongating polypeptide chains.

In eubacteria, this task is performed by a ribosome-binding chaperone called trigger factor — eubacteria being the largest group of bacterial species, and the only subgroup that contains trigger factor. On page 455 of this issue, Kaiser *et al.*[2] describe how they have watched trigger factor at work, providing insight into its protective mechanism (Fig. 1, overleaf).

Hints of how trigger factor might operate have come from static binding experiments and crystal structures[3–5]. By contrast, Kaiser *et al.* used an ingenious fluorescence spectroscopy technique to follow trigger factor dynamically as it interacted with the ribosome and a nascent polypeptide. Consistent with the crystal structures of physiologically meaningful complexes[4,5], they found that when trigger factor binds to the ribosome it undergoes conformational rearrangements and acquires an 'open' activated form that can adhere to hydrophobic patches on the polypeptide. Furthermore, in cases when many hydrophobic segments are exposed, the activated trigger-factor molecule may remain associated with the elongating nascent chain even after the polypeptide has left the ribosome, permitting another molecule of trigger factor to dock on the ribosome. So, until it reaches the next chaperone, the emerging protein may be protected by one or more trigger-factor molecules, with the segments between them probably being protected from degradation because they are partially folded or because of steric hindrance.

This dynamic view is consistent with the fact that all cells contain a significant excess of trigger factor over the number of ribosomes, even though in the cytosol trigger factor binds to almost all active ribosomes at a 1:1 ratio[6]. This means that there is a continuous supply of trigger factor to protect a nascent chain. In the cytosol, unbound trigger-factor molecules exist mainly in pairs; however, they bind to the ribosome singly, contrary to a previous suggestion[7]. The equilibrium between pairs and single molecules might be used to control the amount of readily available trigger-factor molecules for

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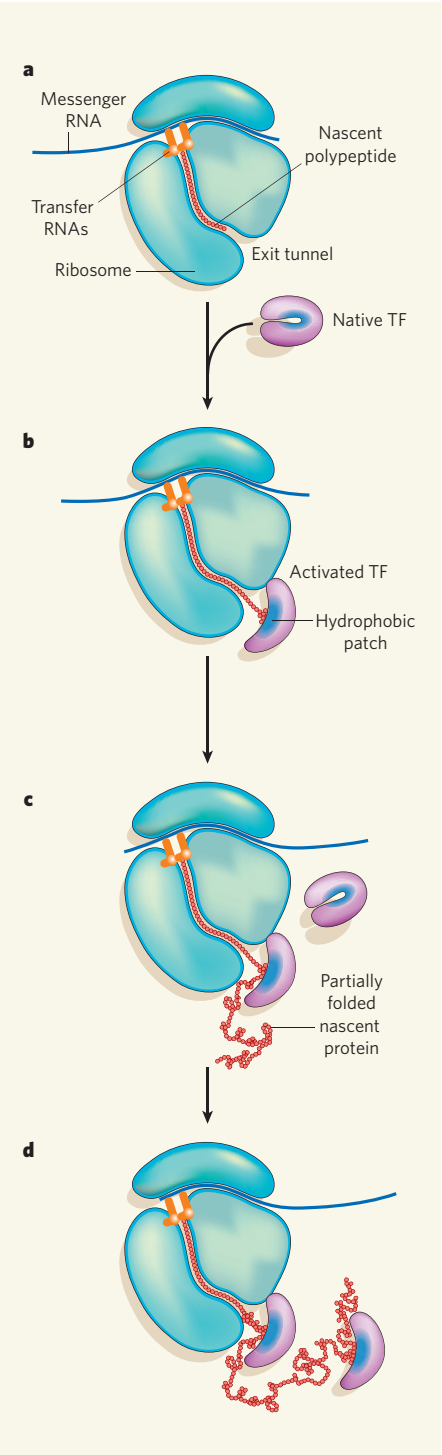

Figure 1 | Trigger factor and protein production by eubacteria[2]. **a**, The code in messenger RNA is read by the ribosome, with transfer RNAs bound to the correct amino acids to form the nascent polypeptide chain. **b**, As the polypeptide emerges from the exit tunnel, trigger factor (TF) in the cytosol is activated and changes conformation; it docks on the ribosome, produces a partially closed shelter, and binds hydrophobic patches of emerging protein chain, preventing misfolding. **c**, **d**, Activated TF can remain associated with the nascent protein even after it has left the ribosome, permitting another TF molecule to dock on the ribosome. Partial folding of the protein segments between TF molecules may protect them from degradation.

binding at the primary site, the ribosome, and for remaining bound to the growing protein until it detaches from the ribosome.

Although there is no dispute that trigger factor protects nascent chains, there is some controversy concerning its mode of action and its possible additional tasks. One suggestion is that, as well as preventing misfolding, trigger factor functions in a static manner by actively folding proteins within a confined void that is created when it binds to the ribosome[8]. This void has never been observed experimentally. Rather, its existence was proposed by assuming that trigger factor maintains its unbound conformation when bound to the ribosome, based on a study of a chimaeric complex of the large ribosomal subunit from *Haloarcula marismortui*[3] (an archaeon, which does not possess trigger factor) and the partially resolved trigger-factor binding domain (TFa) of the eubacterium *Escherichia coli*.

The mechanistic view presented by Kaiser *et al.*[2] challenges this idea. Kaiser *et al.* argue that binding to the ribosome stabilizes trigger factor in an activated form, which differs from its unbound conformation. Their view agrees with the TFa conformation seen in crystal structures of TFa in complex with the large ribosomal subunit when both are from the same eubacterium, *Deinococcus radiodurans*[4,5]. These structures indicate that TFa undergoes a conformational rearrangement on binding to the ribosome, thereby exposing a sizeable hydrophobic region facing the opening of the ribosomal exit tunnel. So, binding of trigger factor to the ribosome induces the factor to take on the activated conformation necessary for its chaperone task, and the nascent chains are prevented from aggregating by attaching to the competing hydrophobic environment on the chaperone. In this way, the competing set of interactions offered by trigger factor leads to a competition with a positive outcome: elimination of misfolding or aggregation of partially folded, newly born proteins.

The finding that a hydrophobic patch becomes exposed on trigger factor only when it binds to the ribosome, and is buried inside the free trigger factor, is consistent with the low affinity of the free factor for isolated, fully folded, hydrophobic proteins, compared with the high affinity of the ribosome-bound factor for the hydrophobic patches of growing nascent chains.

Encapsulating the emerging nascent chain within a void created by the bound trigger factor, as suggested for the modelled structure of the chimaeric complex[3], presents an additional inconsistency with Kaiser and colleagues' dynamic scheme. The rather strong binding implied by the formation of such a void would hamper the smooth dissociation of the complex of trigger factor and the nascent chain from the ribosome, which is an important element in Kaiser and colleagues' proposed dynamic mechanism. However, in a partially enclosed protective space, as observed in the eubacterial complex[4,5], trigger factor should be loosely bound to the ribosome and therefore can readily detach from it.

How universal is the action of trigger factor? How, for instance, does it compare with the situation in yeast? Although neither of yeast's ribosome-associated chaperones shares any amino-acid sequence similarity with the eubacterial trigger factors, yeast ribosomes can recruit trigger factor through an interaction with the yeast relative of L23, the bacterial ribosomal protein that interacts with trigger factor[9]. Does this finding indicate a common mechanism? As yet, there is no definitive answer to this. Perhaps the fact that trigger factor is exclusive to eubacteria relates to a unique property of the eubacterial protein L23, namely, the extended loop that penetrates deep into the ribosome exit tunnel and exposes a sticky hydrophobic patch on its wall. The tip of this loop might undergo conformational rearrangements when TFa binds to the ribosome[10]. Hence, in eubacteria, protein L23 seems to have a key role not only in trigger-factor binding, but also in the dynamic control of the nascent protein.

Further questions include whether trigger factor has additional functions, and what the conformational state of the emerging nascent chain is when it enters trigger factor's shelter, as it may already be partially folded[11]. If it is, does this mean that ribosomes possess inherent chaperone activity themselves? Partial answers are available[12], but much work is required before we have the full picture. ■

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Correction

In the News & Views article "Climate change: The north–south connection" by Eric J. Steig (*Nature* **444,** 152–153; 2006), there was an erroneous temperature inversion. The correct statement is that "there is a strong linear relationship between the magnitude of warming in Antarctica and the duration of the cold period that precedes each abrupt warming event in Greenland." And in the following passage, "The authors' explanation is simple: the duration of the warm periods in Greenland reflects the duration of reduced MOC, and hence the amount of heat retained in the Southern Ocean", "warm" should read "cold".