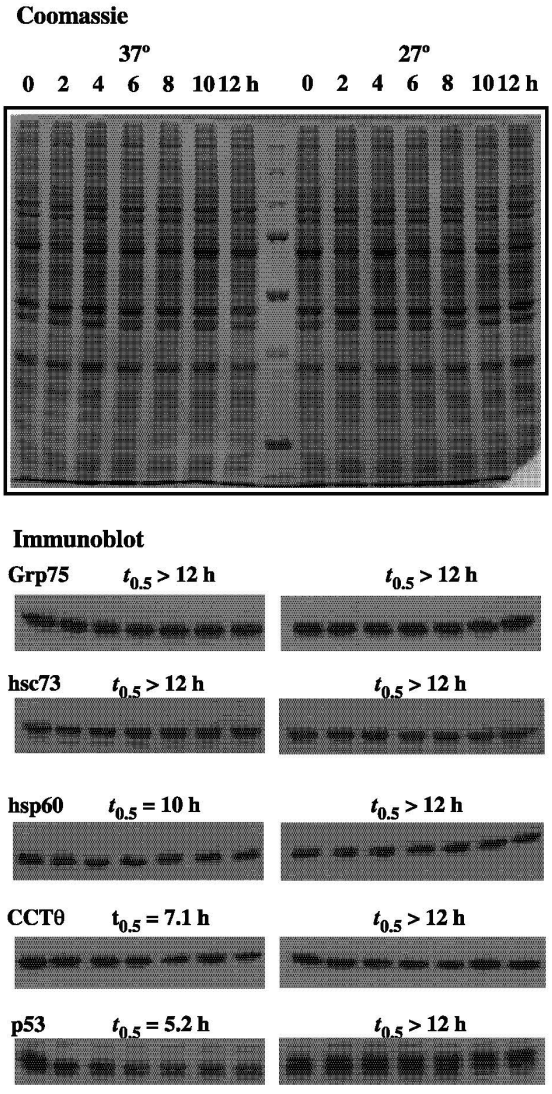

Fig. 6. The half-life of proteins is increased upon exposure to mild cold stress. CHOK1 cells maintained at 37 or 27 °C were then exposed to growth medium containing 50 μg·mL^{-1} cycloheximide. At the indicated times, cells were extracted, and 20 μg of protein was resolved by SDS/PAGE, and then detected either by Coomassie stain (upper panel) or by probing immunoblots for the indicated proteins (lower panel). Molecular mass markers as in Fig. 4.

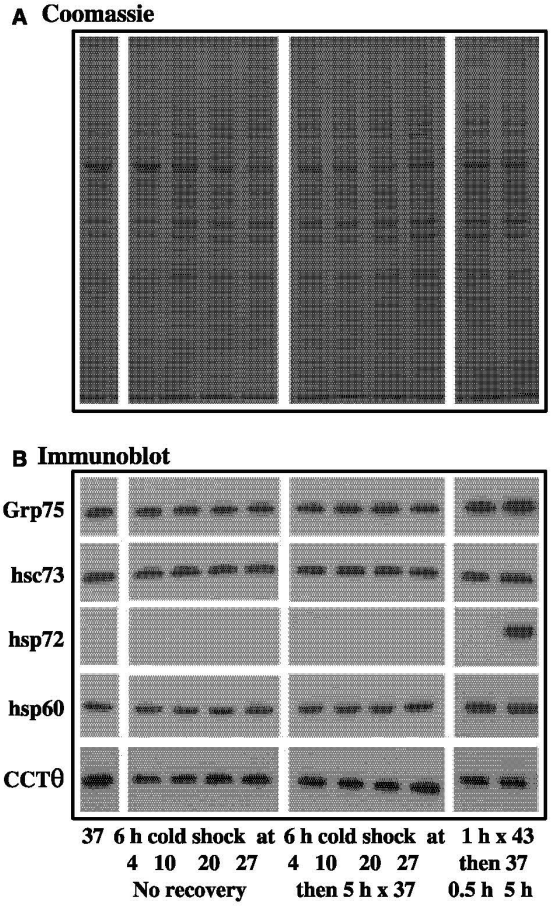

Fig. 7. Marked changes in synthesis rate do not correlate with large changes in overall amounts of relatively abundant proteins. CHOK1 cells maintained at 37 °C, or exposed to the indicated temperature changes, were extracted, and 20 μg of protein was resolved by SDS/PAGE and then detected by Coomassie stain (A) or by probing immunoblots for the indicated proteins (B).

a different mechanism from that involved during a 'classical' recovery from heat shock, during which the inducible form of Hsp70 is robustly expressed.

Transcription of inducible heat shock genes is activated by the binding of heat shock factors (HSFs) to heat shock elements in their promoter-proximal regions [18,19], the best understood being that of HSF1. In unstressed cells, HSF1 exists as a constitutively phosphorylated monomer in the cytoplasm, but during heat stress, HSF1 undergoes trimerization [20] and becomes hyperphosphorylated [21]. It is this hyperphosphorylated, trimeric form that accumulates in the nucleus and binds to heat shock elements, thereby activating transcription [21]. Figure 9B shows the basal level of constitutive phosphorylation of HSF1 determined using immunoblots of HSF1 in cell extracts prepared in the presence of protein phosphatase inhibitors (Fig. 9B, as a cluster of bands ~ 85–90 kDa). The hyperphosphorylation occurring during heat shock could also be readily demonstrated (Fig. 9B). Cold shock produced a much more subtle change in the HSF1 banding pattern, evident immediately after cold shock and then slowly returning to the

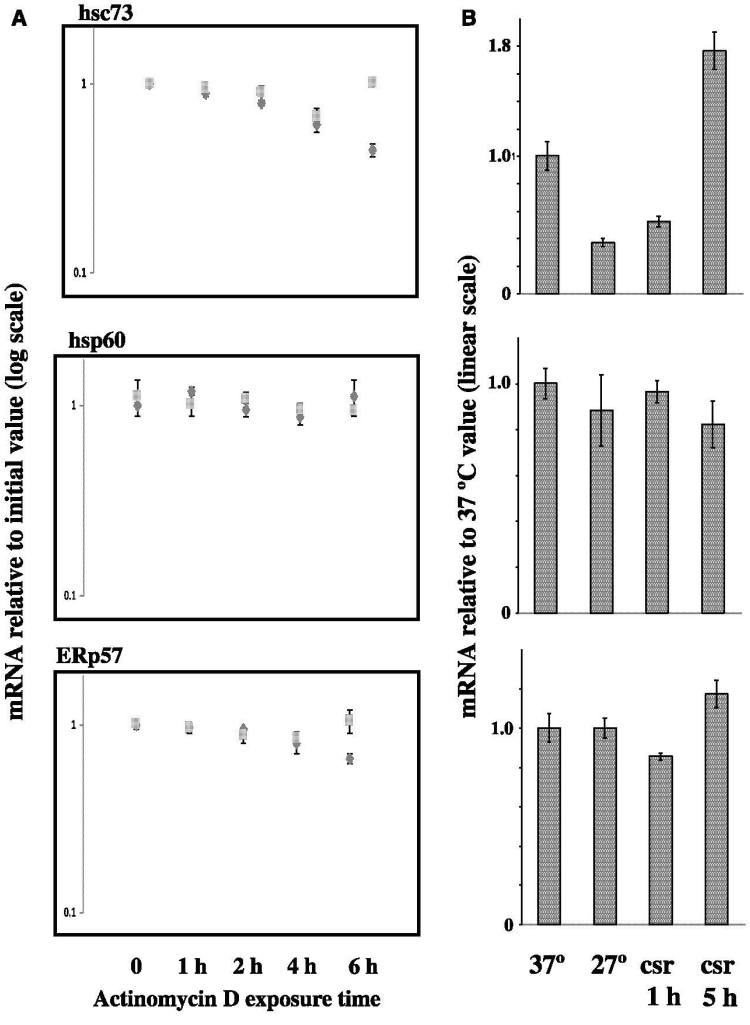

Fig. 8. Specific mRNAs are longer-lived at 27 °C than at 37 °C. (A) CHOK1 cells maintained at 37 °C (squares) or 27 °C (diamonds) were then exposed to growth medium containing 2 $\mu g \cdot mL^{-1}$ actinomycin D at the same temperatures. (B) CHOK1 cells maintained at 37 °C or held at 27 °C for 6 h without or with a recovery period (crs, cold shock recovery) at 37 °C for 1 h or 5 h. At the indicated times, total RNA was extracted from the cells and the indicated mRNAs were quantified by qRT-PCR. Data are normalized to the initial mRNA content at 37 or 27 °C.

constitutive pattern during a subsequent 5 h recovery. This cold shock-induced phosphorylation change in HSF1 was more pronounced with increasing hypothermia, and was most evident in the very cold-sensitive P19 cells.

Trimerization was assessed by chemical cross-linking analysis, using ethylene glycol *bis*(succinimidylsuccinate), to stabilize the trimer for SDS/PAGE resolution prior to immunoblot detection of HSF1. Heat shock-induced trimerization of HSF1, i.e. the hyperthermic response, was extensive, so that immediately after heat shock, almost all HSF1 was in the hyperphosphorylated, trimeric form (Fig. 9C, lower panel). In contrast, little trimeric HSF1 was evident immediately after cold (hypothermic) shock, and only modest amounts were present during recovery from this cold stress, even though the synthesis rates of constitutive heat shock proteins were increased at this time. Once again, although this response was stronger in the most cold-sensitive cell line, P19, it was still weak in comparison to that observed upon heat stress. These findings collectively suggest that the recovery from cold stress, at least in rodent cells, does not initiate a classical heat shock response, and that any response initiated through HSF1 is comparatively weak or restricted in comparison to a classical heat shock response.

Discussion

Here we report changes in the cellular architecture, and the synthesis and degradation rates, of specific

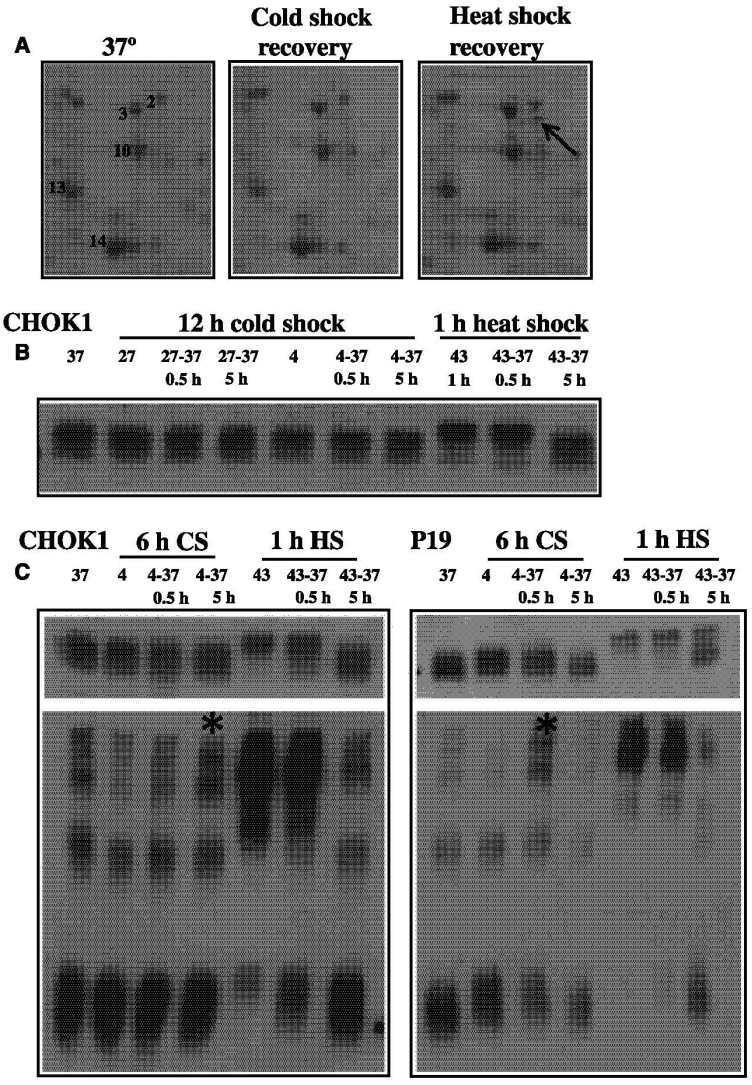

Fig. 9. A classical heat shock response is not initiated upon recovery of cold-stressed cells at 37 °C. (A) Proteins extracted from CHOK1 cells that had been maintained at 37 °C, or held at 27 °C for 6 h and then transferred to 37 °C for 5 h (cold shock recovery), or held at 43 °C for 1 h and then transferred to 37 °C for 5 h (heat shock recovery), and then radiolabelled for a further 1 h at 37 °C, were resolved and detected as in Fig. 5. Only the area including Grp75 (spot 2) to actin (spot 14) is shown; the spot numbers refer to the proteins listed in Table 2. Hsp72 is arrowed. (B) An immunoblot of proteins extracted from CHOK1 cells maintained at 37 °C, or held at 4 or 27 °C for 12 h, or held at 43 °C for 1 h, with or without subsequent recovery at 37 °C for 0.5 h or 5 h, probed for HSF1. (C) Immunoblots of SDS/PAGE resolutions (upper panels) or of nondenaturing gel resolutions (lower panels) of proteins extracted from CHOK1 and P19 cells maintained at 37 °C, or held at 4 °C for 6 h, or held at 43 °C for 1 h, with or without subsequent recovery at 37 °C for 0.5 h or 5 h, probed for HSF1. Trimerization of HSF1 upon recovery from cold stress is indicated by an asterisk. CS, cold stress; HS, heat stress.

proteins in mammalian cells subjected to both mild and severe cold stress, and during recovery from hypothermic shock. Collectively, they help to define the specific cellular responses and protein players during cold stress and recovery. The changes identified here in the synthesis and turnover rates reveal that adaptations are easy to miss when comparing total protein levels monitored either by densitometry-based studies (typically, global proteomic 'snapshot' studies) or by immunoblot. Our studies have shown that subphysiological temperatures induce specific changes in synthesis rates for proteins involved in a wide spectrum of cellular activities, including energy metabolism, cytoskeletal organization, protein synthesis, purine biosynthesis, secretion and, most particularly, molecular chaperone function.

Representative molecular chaperones from three intracellular compartments, the cytoplasm, the mitochondrion, and the ER, were all detected as part of the adaptive changes of cells exposed to mild hypothermia and, more especially, in cells recovering from this state. It is of particular interest that the synthesis rates of the cytoplasmic molecular chaperones Hsc73 and HOP/p60, and of the ER chaperone ERp57, were increased upon cold stress at 27 °C but not at 32 °C. The strength of hydrophobic interactions decreases with decreasing temperature, and so higher orders of protein structure become less stable at subphysiological

temperatures [22]. Thus, at 27 °C, this must become problematic and generate unfolding of existing proteins and/or compromise the folding of newly synthesized proteins, as appreciable protein synthesis is still taking place at this temperature. Furthermore, as protein degradation becomes undetectable at 27 °C, the cell responds to the unfolded protein load by increasing the synthesis of selected molecular chaperones to sequester unfolded proteins until more favourable conditions, including revival of turnover apparatus, are restored.

Rapid recovery of protein synthesis capacity upon rewarming after cold stress would be expected to increase the requirement for molecular chaperones involved in protein folding, particularly in the cytoplasm. However, we also observed increases in the synthesis rates of chaperones in the mitochondrion and the ER after restoration to normothermic conditions. This will undoubtedly be, in part, a response to the overall increase in protein synthesis activity, but the fact that two of these chaperones, Grp75 and ERp57, are redox-sensitive chaperones indicates that the resumption of metabolic activity upon rewarming increases the free radical load on the cell, as might be expected. As the synthesis of the mitochondrial chaperones did not increase during cold shock at 27 °C, this further supports the idea that it is a change in the redox state upon rewarming that is the main stimulus for the increased synthesis rate of the mitochondrial chaperones during recovery from cold stress.

During recovery from cold stress, we also detected increased synthesis of several constitutive heat shock proteins but not of the classical heat shock protein, inducible Hsp72. Kaneko *et al.* [23] also reported no increase in Hsp72 mRNA upon rewarming NIH 3T3 cells from 32 to 37 °C. Earlier studies using human cell lines did detect increased amounts of Hsp72 upon rewarming after cold shock [16]. An explanation for this discrepancy is that in human cells, Hsp72 is constitutively expressed, whereas in rodent cell lines it is strictly inducible [17]. It would appear, then, that the heat shock proteins induced during recovery from cold stress are the constitutive heat shock proteins, not the strictly inducible ones. Specifically with regard to heat shock protein induction, our findings show that the HSF1 activation process during recovery from cold stress is different from that induced during the classical heat shock response. The degree of HSF1 hyperphosphorylation varies from robust in the normal heat shock response to only a partial response as reported here for cold shock recovery, but also following exposure to certain antimicrotubule drugs used in cancer chemotherapy [24]. Under these latter circumstances, not only HSF1 hyperphosphorylation but also HSF1 trimerization occurred at a reduced level, and only induction of the constitutive heat shock proteins Grp75 and Hsp60, not of inducible Hsp72, was detected.

It is generally accepted that cold stress results in the attenuation of mRNA translation, although we show here that at mildly hypothermic temperatures (27 and 37 °C), protein synthesis is active, although reduced, and that both the banding pattern and relative intensity of polypeptides synthesized at these lower temperatures remain very similar to those observed at 37 °C. Translation is a tightly controlled process, modulated greatly by the (de)phosphorylation of key initiation and elongation factors. Previous studies have shown that mutant initiation factors can elevate the effects of such a slowdown in mRNA translation upon cold stress [25]. Here, we observed that cold stress at 32 °C results in reduced levels of newly synthesized eIF3i, a subunit of initiation factor 3. Upon recovery, this is reversed and eIF3i levels are increased. Although eIF3i is essential for mRNA translation *in vivo* [26–28], it is not essential for the reconstruction of initiation complexes that can scan and find the AUG start codon [29]. Therefore, its role *in vivo* is likely to be related to regulation of initiation. Furthermore, overexpression of eIF3i has been shown to be associated with increased cell proliferation, an accelerated cell cycle, and an increase in cell size, whereas the knockdown (by RNA interference) of eIF3i resulted in the reverse of these effects [30,31]. These opposing consequences of eIF3i knockdown or overexpression are mirrored in the observations here of the cellular responses to cold stress at 32 °C and recovery, respectively. It is therefore likely that eIF3i plays a pivotal role in directing cell growth and proliferation upon cold stress and subsequent recovery.

It has been reported elsewhere that cells cultivated under mildly hypothermic conditions undergo cell cycle arrest, predominantly in G_1, but also in G_2/M [32], and it has recently been suggested that this is in part due to expression of the RNA-binding cold shock proteins Cirp and Rbm3, as their overexpression under normothermic conditions can lead to cell cycle arrest [9]. Previous reports, however, have shown that p53-deficient mammalian cells do not show cell cycle arrest at mildly hypothermic temperatures [33,34], and that at 4–20 °C, p53 induces p21 (WAF1) expression [34]. Our results support this mechanism of p53-mediated cell cycle arrest. p53 in CHOK1 cells has a point mutation that confers unusual stability on this protein and prevents these cells undergoing a normal response to DNA damage, i.e. induction of p21 and consequent cell cycle arrest in G_1 [15]. Nevertheless, during mild cold stress, we observed

an increase in the level of p53 in CHOK1 cells, a change in p53 isoform pattern due to post-translational modification, and induction of p21 expression. Furthermore, re-entry of cells into the cell cycle upon return to normothermic conditions could be mediated by the increased synthesis of Grp75 that we observed under these conditions. Expression of Grp75 has a two-fold positive effect on cell cycle progression. When present in the cytoplasm, it sequesters p53 [35], thereby preventing entry into the nucleus and subsequent activation of p21 transcription. Furthermore, p53 binding to the centrosome [36,37], which is inhibitory to centrosome duplication, is antagonized by Grp75 [38]. Additionally, Grp75 itself binds to the centrosome, thereby activating Mps1 protein kinase, the activity of which is essential for the initiation of centrosome duplication [39]. Under this model, cell cycle arrest upon cold stress and then re-entry upon recovery is modulated and controlled via the balance of p53 and Grp75 levels.

Finally, our electron microscopy studies and Oil Red O staining show the presence of lipid-containing vesicle-type structures under conditions of severe cold stress. These vesicle-like structures may be the result of lipid material being secreted from the cell, or alternatively, these vesicles may only be observed under severe cold stress because the membrane rigidity and/or membrane-associated cell functions are so severely compromised at very low temperatures that this results in the arrest of the vesicles before secretion, whereas at higher temperatures these are secreted efficiently and hence not observed. It is well known that cold stress results in membrane rearrangements [40], and changes in cellular lipids have been linked to the heat shock response in yeast [41]. More recent research has shown that changes in the lipid composition of the cell membrane induce the phosphorylation of p53 by the ataxia–telangiectasia and Rad-3 related kinase [42], and we are now investigating whether cold stress-induced cell cycle arrest is due to this ataxia–telangiectasia and Rad-3 related kinase activation of the p53–p21 signalling pathway.

In conclusion, we have here identified a number of mechanisms involved in the response of *in vitro* cultured mammalian cells to mild and severe cold stress, and in recovery from such stress. In addition to a global decrease in mRNA and protein turnover, the synthesis of specific proteins involved in regulating cell growth, proliferation and mRNA translation are upregulated or downregulated during cold stress and recovery. Furthermore, changes in the lipid composition of the cell may underpin these responses, especially upon severe cold stress. On the basis of the results presented here, we suggest that the cytoskeleton, and the balance in the levels of p53, Grp75 and eIF3i, are likely to be of particular importance during the response to, and recovery from, cold stress that allows mammalian cells to survive and recover from low-temperature stress.

Experimental procedures

Cell lines, routine culture conditions, and treatment conditions

CHOK1 cells were sourced from the European Collection of Cell Cultures and P19 cells from P. Andrews, University of Sheffield, UK. Cells were routinely cultured in DMEM/F12 (Invitrogen, Paisley, UK) supplemented with 200 mM L-glutamine, 500 μM glutamic acid, 500 μM asparagine, 30 μM adenosine, 30 μM guanosine, 30 μM cytidine, 30 μM uridine, 10 μM thymidine, 1% nonessential amino acids (Invitrogen, Paisley, UK), and 10% (v/v) heat-inactivated fetal bovine serum (PAA Laboratories Ltd, Yeovil, UK) at 37 °C in a 5% CO_2 atmosphere. NIH 3T3 cells were also sourced from the European Collection of Cell Cultures and maintained as above, except that DMEM was used in place of DMEM/F12. For radiolabelling, the routine maintenance media were replaced with cysteine/methionine-deficient DMEM (Sigma-Aldrich, Poole, UK) supplemented with 10% (v/v) dialysed, heat-inactivated fetal bovine serum, 2 mM glutamine and 1770 kBq·mL^{-1} Pro-Mix L-[^{35}S] cell labelling mix (GE Healthcare, Chalfont St Giles, UK), and then incubated for 1 h at the indicated temperature. Uptake and incorporation of the ^{35}S-labelled amino acids was as previously described [43]. Cold shock was undertaken in routine medium for 6 h or 30 h at 4, 10, 20, 27 and 32 °C in appropriately regulated incubators. Heat shock was also undertaken in the routine culture medium for 1 h by flotation in a water bath at 43 °C. Treatment of cells with the antimicrotubule drug nocodazole was performed in routine medium at 1–3 μg·mL^{-1} for 2 h at 37 °C. Recovery incubations were undertaken in routine culture medium at 37 °C for 0.5, 1.5 and 5 h. For the determination of mRNA half-lives, cells were incubated in routine culture medium containing 2 μg·mL^{-1} actinomycin D. For protein half-life determinations, cells were incubated in routine culture medium containing 50 μg·mL^{-1} cycloheximide.

Extraction of RNA and protein from cell pellets

Total RNA was prepared from intact cells using the commercially available RNeasy kit (Qiagen). Cell extracts for protein analyses were prepared by lysing cells into ice-cold extraction buffer [20 mM Hepes/NaOH, pH 7.2, containing 100 mM NaCl, 1% (w/v) Triton X-100, protease inhibitors (10 μL·mL^{-1} leupeptin, 2 μg·mL^{-1} pepstatin, 0.2 mM phen-

ylmethanesulfonyl fluoride) and protein phosphatase inhibitors (50 mM NaF, 1 mM activated Na_3VO_4)]. Cell lysates were then centrifuged at 16 000 ***g*** for 2 min at 4 °C, and the resulting supernatants were retained for further analysis. For the determination and detection of HSF1 trimer formation levels, cell extracts were cross-linked with ethylene glycol *bis*(succinimidylsuccinate) (Sigma) at room temperature for 30 min, and then blocked with 50 mM Tris/HCl (pH 7.5) at room temperature for 15 min.

Gel electrophoresis analysis of protein extracts

For SDS/PAGE analysis, 10% separation gels were utilized according to the procedure of Laemmli [44]. Prior to NEPHGE-SDS/PAGE, the proteins in cell extracts were precipitated overnight with four volumes of acetone at −20 °C. Following NEPHGE-SDS/PAGE, resolved proteins were detected by Coomassie staining and/or autoradiography using Hyperfilm MP (GE). Gel images were analysed using the commercially available PROGENESIS PG200 software package (Nonlinear Dynamics, Newcastle-upon-Tyne, UK) to determine spots that had changed in abundance. Spot detection was undertaken using the spot detection wizard with the parameters set as follows: minimum spot area, 16; split factor, 7; peak location, use centre of mass as peak. Manual splitting of nonsplit spots and deletion of noise were then undertaken. Following spot detection, background subtraction was achieved using the mode of nonspot option with a margin of 45. In-gel tryptic digestion of excised spots and protein identification by MALDI-TOF MS were undertaken according to Smales *et al.* [45]. Analysis was undertaken on triplicate biological samples, and only spots whose abundance was changed at the 95% confidence level ($P < 0.05$) relative to the 37 °C control were considered to show significant changes in polypeptide synthesis rates.

Determination of mRNA levels by qRT-PCR

qRT-PCR was used to determine the relative mRNA levels of target genes using the commercially available BioRad iScript qRT-PCR kit according to the manufacturer's instructions, and the appropriate primers to amplify CHO sequences as listed in Table 2. All reactions were performed using a BioRad DNA Engine Chromo4 Continuous Fluorescence Detector thermocycler (BioRad, Hemel Hempstead, UK). Cycling conditions included a reverse transcription step by incubation at 50 °C for 20 min, followed by heating at 95 °C for 15 min. Sequentially, the target templates were amplified using 39 cycles (30 s at 98 °C, 15 s at 55 °C). The fluorescence threshold value (C_t) was calculated using OPTICON MONITOR software (version 3.1; BioRad). For normalization purposes, all levels were normalized to control levels at 37 °C.

Table 2. Primers used for qRT-PCR experiments described in this article.

Name	Sequence (5′- to -3′)
Hsc73F	CGACAAGAAGGACATCAGCGAG
Hsc73R	GAATCGAGCACGGGTAATGGAG
Hsp60F	TGCTCATCGTAAGCCCTTGGTC
Hsp60R	TTCTCCAAACACCGCACCAC
ERp57F	AACTACAGATTTGCACACACC
ERp57R	CAGTATATACCACAGTTTTGTC

Immunoblot analysis

PAGE-resolved polypeptides were transferred to nitrocellulose using standard procedures, and then blocked with 5% (w/v) nonfat milk in NaCl/P_i or for phosphorylation-dependent epitopes in 0.2% (w/v) Tween-20. Antibody probes against α-tubulin (TAT) and β-tubulin (KMX) [46] were gifts from K. Gull (University of Oxford, UK), and the antibody 23c against STOP [13] was a gift from C. Bosc and D. Job (Commisariat A L'Energie Atomique, Grenoble, France). Affinity-purified rabbit polyclonal antibodies against the C-termini of CCT subunits and against Hsc70 were as described elsewhere [47]. Commercial antibodies against Grp75 (clone 30A5), Hsp60 (clone LK-2) and HSF1 (rabbit polyclonal) were from Stressgen, antibody against p53 (clone DO-7) was from Dako, and antibody against p21 was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Peroxidase-conjugated secondary antibodies were detected by enhanced chemiluminescence using Hyperfilm ECL (GE). Images were analysed using KODAK GEL LOGIC 100 imaging system software. The linearity of antibody response over the concentration range of target protein used is shown in Fig. S4.

Immunofluorescence microscopy

For immunofluorescence microscopy studies, cells were grown on 13 mm glass coverslips and then fixed with methanol at −20 °C for 5 min, or with 4% (w/v) paraformaldehyde in NaCl/P_i; this was followed by permeabilization with 0.1% (w/v) Triton X-100 in NaCl/P_i. Cells were rehydrated after methanol fixation for 5 min in NaCl/P_i. All coverslips were then blocked for 15–30 min in 3% (w/v) BSA in NaCl/P_i. Incubation with primary antibodies diluted in blocking solution (TAT, 1 : 100; anti-hsp60, 1 : 100) was performed overnight at 4 °C. The appropriate secondary antibodies (anti-mouse tetramethyl rhodamine iso-thiocyanate; Sigma) were diluted 1 : 100 before use. F-actin staining with rhodamine–phalloidin (Molecular Probes, Invitrogen, Paisley, UK) was achieved according to the manufacturer's instructions. Cells were counterstained with 4′,6-diamidino-2-phenylindole, and coverslips were then mounted in

Mowiol containing *p*-phenylenediamine as antifade. Cells were then examined under a Leica DMR fluorescence microscope, and images were captured with a Leica DC300F digital camera.

Electron microscopy

For electron microscopy cells, were grown at 37 °C or in the cold as described, and then fixed with 2.5% glutaraldehyde in NaCl/P_i, postfixed with 1% osmium tetroxide, and dehydrated with a graded series of alcohols. After two changes of 100% ethanol, they were detached from the flasks by agitation in ethoxypropane, and then embedded in Agar Low Viscosity Resin. Sections were cut at 60–90 nm, stained with uranyl acetate and lead citrate, and examined in a Jeol 1230 transmission electron microscope (Jeol UK, Welwyn Garden City, UK) operating at 80 kV. Images were recorded with a Gatan Multiscan 600CW camera (Gatan UK, Oxford, UK).

Acknowledgements

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Supporting information

The following supplementary material is available:

Fig. S1. The small vesicles associated with severely cold-stressed cells do not stain for protein or RNA but do stain for uncharged lipid.

Fig. S2. (A) Mild hypothermia does not initiate apoptosis in CHOK1 or P19 cells. (B) Mild hypothermia is not associated with acetylation of p53.

Fig. S3. Protein synthesis in P19 and 3T3 cells exposed to, and recovering from, cold stress.

Fig. S4. Antibody response to a concentration range of proteins identified by immunoblot.

This supplementary material can be found in the online version of this article.

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BIOCHEMICAL INSIGHTS INTO THE MECHANISMS CENTRAL TO THE RESPONSE OF MAMMALIAN CELLS TO COLD-STRESS AND SUBSEQUENT REWARMING

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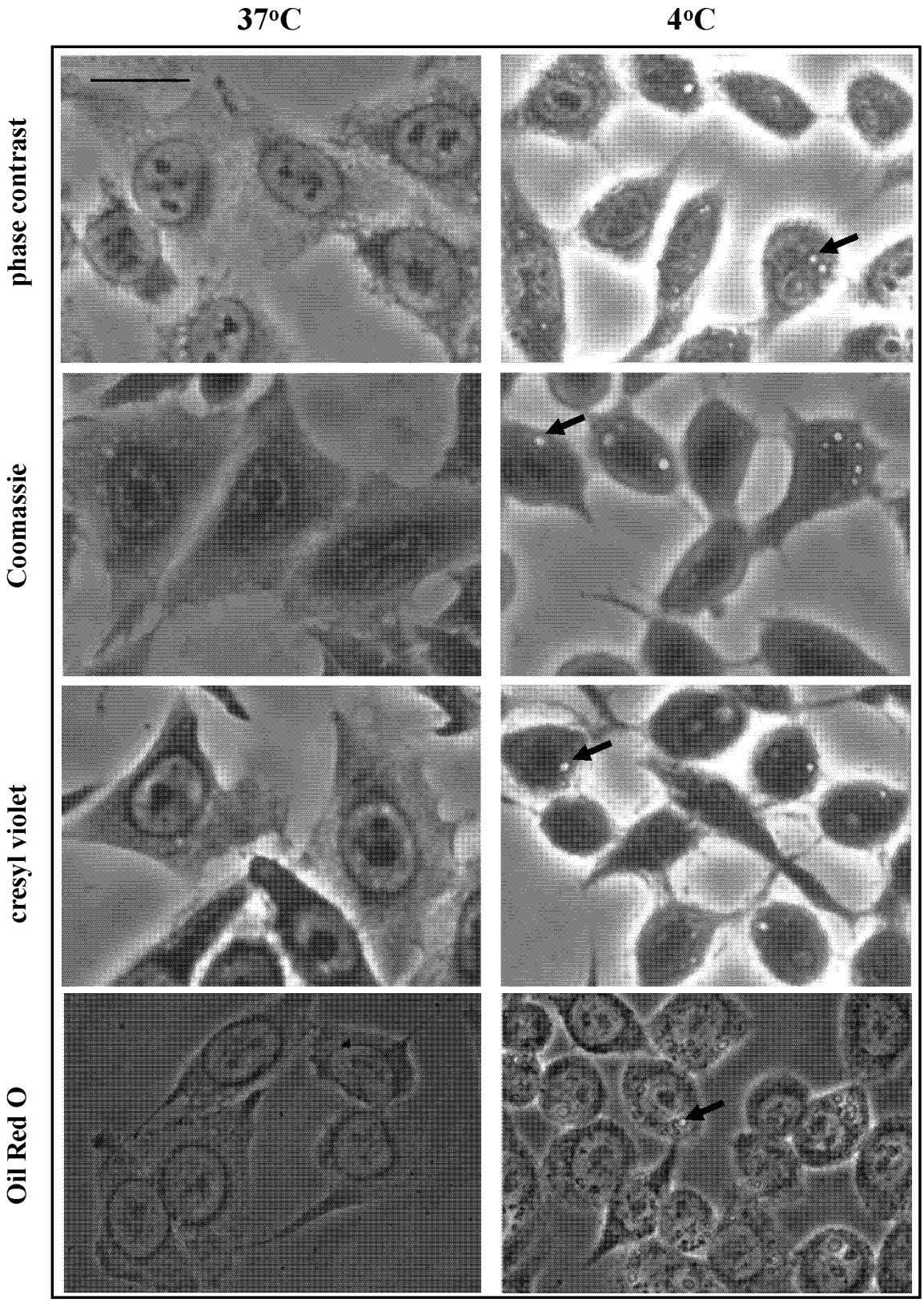
Titles of Supplementary Material

Supplementary FIGURE S1. The small vesicles associated with severely cold-stressed cells do not stain for protein or RNA but do stain for uncharged lipid. CHOK1 cells, growing on 13 mm glass coverslips, were maintained at 37°C or transferred to 4°C for 6 h prior to fixation with -20°C methanol, then left unstained (phase contrast) or stained with 0.1% (w/v) Coomassie blue G250 in 50% methanol-10% acetic acid, or with 0.5% (w/v) cresyl violet acetate in H_2O, both for 2 min or with a saturated solution of Oil Red O in 60% (v/v) isopropanol for 10 min, before washing with phosphate buffered saline or, in the case of Oil Red O, first with 60% isopropanol and then with water. Arrows point to the vesicles observed at 4°C. Bar = 10 μm.

Supplementary FIGURE S2. (A) Mild hypothermia does not initiate apoptosis in CHOK1 or P19 cells. Cells were maintained at 37°C for 24 h then transferred to the indicated temperatures. At the indicated times, cells were washed once with PBS then lysed into SDS-PAGE sample buffer. Lysates were passaged 6 times through a 19 gauge syringe needle then held at 100°C for 2 min. Immunoblots of 20 μg samples resolved over 10% resolution gels were probed with anti-PARP rabbit polyclonal (Stressgen AAP-250). Note: Early apoptosis is characterized by the appearance of an 85 kDa cleavage product of PARP which is absent in the blots. **(B) Mild hypothermia is not associated with acetylation of p53.** Immunoblots of cells treated as in (A), or extracted into a buffer containing the deacetylase inhibitors trichostatin A (1 μM) and nicotinamide (5 mM, not shown) were probed for acetylated p53 (Cell Signaling #2570). Note: Increased acetylation of p53 was detected as cells became confluent as exemplified by the later time points for cells held at 37°C and 32°C.

Supplementary FIGURE S3. Protein synthesis in P19 and 3T3 cells exposed to, and recovering from, cold-stress. Cells maintained at 37°C, or then exposed to the indicated temperatures for the indicated times, were radiolabelled in a methionine-cysteine deficient growth medium supplemented with 1770 kBq/ml [^{35}S] methionine-cysteine cell labelling mix for 1 further hour at the indicated temperature. 30 μg of extracted proteins were resolved by SDS-PAGE and detected by autoradiography. MW markers were 205, 150, 100, 75, 50, 37, 25, 20 and 15 kDa.

Supplementary FIGURE S4. Antibody response to a concentration range of proteins identified by immunoblot. (A) 30 – 1 μg of proteins extracted from CHOK1 cells were resolved by SDS-PAGE and detected by immunoblot with the indicated antibodies. (B) shows the densitometric analysis of the data in (A).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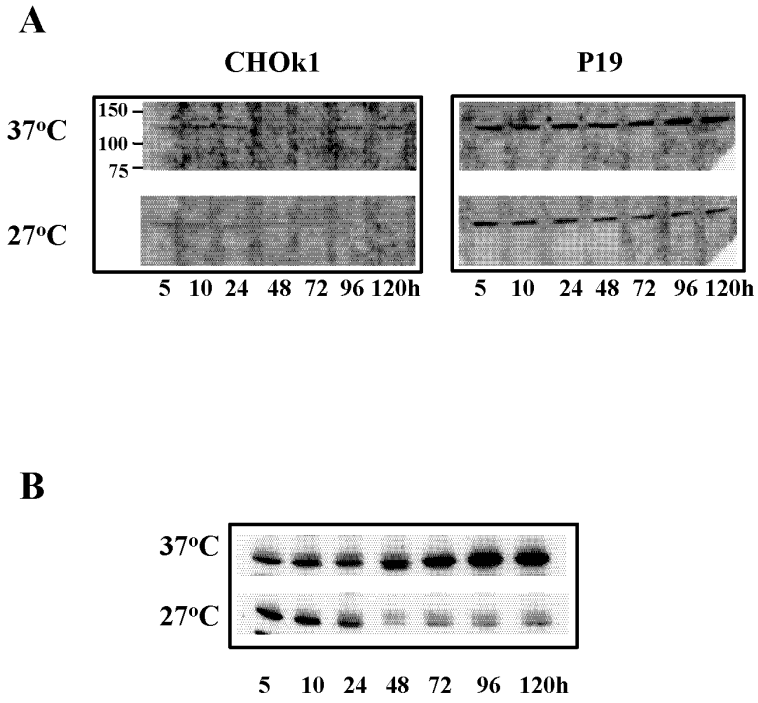
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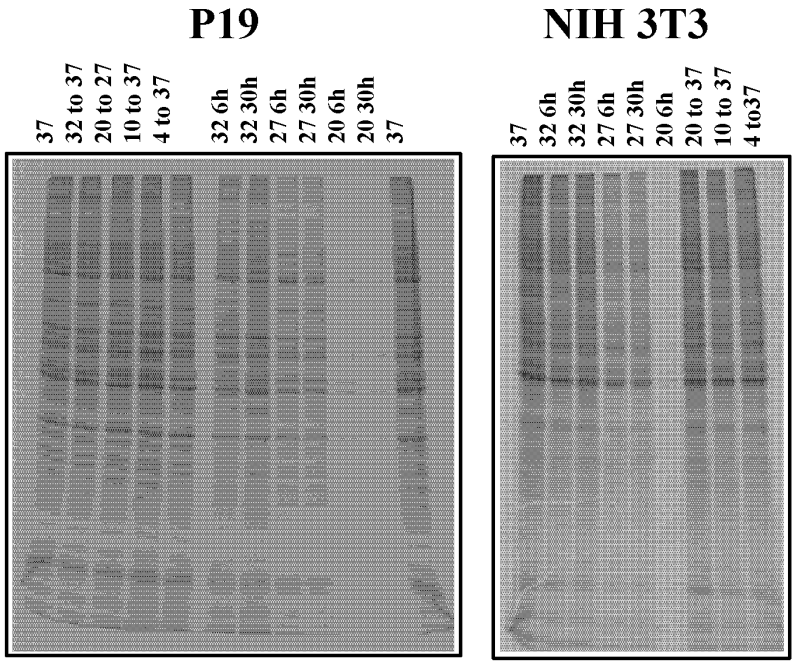
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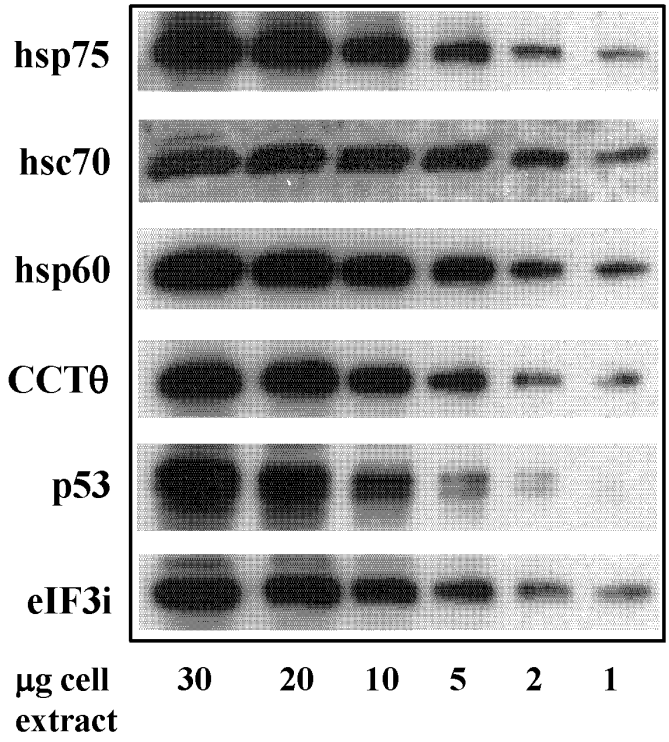
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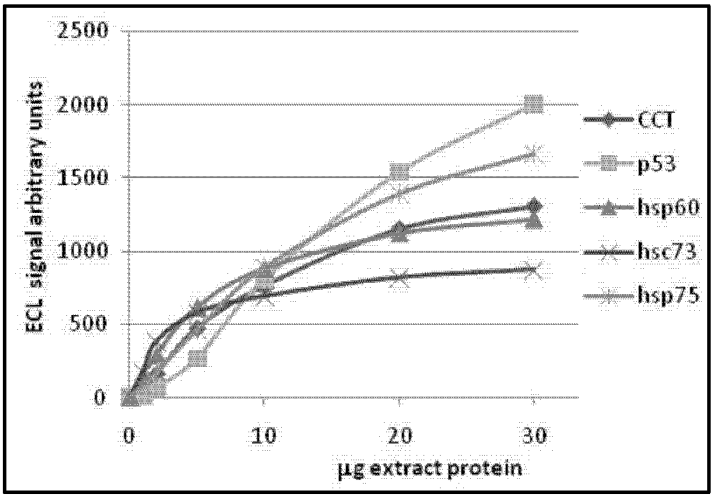
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McGRAW-HILL DICTIONARY OF SCIENTIFIC AND TECHNICAL TERMS

Sixth Edition

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On the cover: Representation of a fullerene molecule with a noble gas atom trapped inside. At the Permian-Triassic sedimentary boundary the noble gases helium and argon have been found trapped inside fullerenes. They exhibit isotope ratios quite similar to those found in meterorites, suggesting that a fireball meteorite or asteroid exploded when it hit the Earth, causing major changes in the environment. ***(Image copyright © Dr. Luann Becker. Reproduced with permission.)***

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FENNEL

Fennel (*Foeniculum vulgare*). (*USDA*)

fenestrated membrane [HISTOL] One of the layers of elastic tissue in the tunica media and tunica intima of large arteries. { 'fen·ə,strād·əd 'mem,brān }

fenestration [ARCH] The arrangement of openings, especially windows, in the wall of a building. [BIOL] **1.** A transparent or windowlike break or opening in the surface. **2.** The presence of windowlike openings. { ,fen·ə'strā·shən }

fenitrothion [ORG CHEM] $C_9H_{12}NO_5PS$ A yellow-brown liquid, insoluble in water; used as a miticide and insecticide for rice, orchards, vegetables, cereals, and cotton, and for fly and mosquito control. { ,fen·ə·trō'thī,ān }

fennel [BOT] *Foeniculum vulgare*. A tall perennial herb of the family Umbelliferae; a spice is derived from the fruit. { 'fen·əl }

fennel oil [MATER] The essential oil obtained from fennel; a colorless liquid with aromatic scent and bitter taste, insoluble in water and boiling at 160–220°C; used in medicine, perfumes, and liqueurs. Also known as oil of fennel. { 'fen·əl ,ȯil }

fen peat *See* low-moor peat. { 'fen ,pēt }

Fenske equation *See* Fenske-Underwood equation. { 'fen·skē i,kwā·zhən }

Fenske-Underwood equation [CHEM ENG] Equation in plate-to-plate distillation-column calculations relating the number of theoretical plates needed at total reflux to overall relative volatility and the liquid-vapor composition ratios on upper and lower plates. Also known as Fenske equation. { ¦fen·skē 'ən·dər,wůd i,kwā·zhən }

fenster *See* window. { 'fen·stər }

fensulfothion [ORG CHEM] $C_{11}H_{17}S_2O_2P$ A brown liquid with a boiling point of 138–141°C; used as an insecticide and nematicide in soils. { ,fen,səl·fō'thī,ān }

fentinacetate [ORG CHEM] $C_{20}H_{18}O_2Sn$ A yellow to brown, crystalline solid that melts at 124–125°C; used as a fungicide, molluscicide, and algicide for early and late blight on potatoes, sugarbeets, peanuts, and coffee. Also known as triphenyltinacetate. { ,fent·ən'as·ə,tāt }

fenuron [ORG CHEM] $C_9H_{12}N_2O$ A white, crystalline compound with a melting point of 133–134°C; soluble in water; used as a herbicide to kill weeds and bushes. { ,fen'yů,rān }

fenuron-TCA [ORG CHEM] $C_{11}H_{13}Cl_3N_2O_3$ A white, crystalline compound with a melting point of 65–68°C; moderately soluble in water; used as a herbicide for noncrop areas. { ,fen'yů,rān ¦tē¦sē¦ā }

FEP resin *See* fluorinated ethylene propylene resin. { ¦ef¦ē¦pē 'rez·ən }

ferbam [ORG CHEM] $C_9H_{18}FeN_3S_6$ [iron(III) dimethyldithiocarbamate] A fungicide for protecting fruits, vegetables, melons, and ornamental plants. { 'fər·bəm }

ferberite [MINERAL] $FeNO_4$ A black mineral of the wolframite solid-solution series occurring as monoclinic, prismatic crystals and having a submetallic luster; hardness is 4.5 on Mohs scale, and specific gravity is 7.5. { 'fər·bə,rīt }

ferghanite [MINERAL] $U_3(VO_4)_2·6H_2O$ Sulfur-yellow mineral composed of hydrated uranium vanadate, occurring in scales. { fər'gä,nīt }

fergusonite [MINERAL] $Y_2O_3·(Nb,Ta)_2O_5$ Brownish-black rare-earth mineral with a tetragonal crystal form; it is isomorphous with formanite. { 'fər·gə·sə,nīt }

Fermat numbers [MATH] The numbers of the form $F_n = (2^{(2^n)}) + 1$ for $n = 0, 1, 2, \ldots$. { 'fer·mä ,nəm·bərz }

Fermat's last theorem [MATH] The proposition, proven in 1995, that there are no positive integer solutions of the equation $x^n + y^n = z^n$ for $n \geq 3$. { fer'mäz ¦last 'thir·əm }

Fermat's principle [OPTICS] The principle that an electromagnetic wave will take a path that involves the least travel time when propagating between two points. Also known as least-time principle; stationary time principle. { fer'mäz 'prin·sə·pəl }

Fermat's spiral [MATH] A plane curve whose equation in polar coordinates (r,θ) is $r^2=a^2\theta$, where a is a constant. { fer'mäz ,spī·rəl }

Fermat's theorem [MATH] The proposition that, if p is a prime number and a is a positive integer which is not divisible by p, then $a^{p-1} - 1$ is divisible by p. { 'fer,mäz ,thir·əm }

ferment [BIOCHEM] An agent that can initiate fermentation and other metabolic processes. { ¦fər¦ment }

fermentation [MICROBIO] An enzymatic transformation of organic substrates, especially carbohydrates, generally accompanied by the evolution of gas; a physiological counterpart of oxidation, permitting certain organisms to live and grow in the absence of air; used in various industrial processes for the manufacture of products such as alcohols, acids, and cheese by the action of yeasts, molds, and bacteria; alcoholic fermentation is the best-known example. Also known as zymosis. { ,fər·mən'tā·shən }

fermentation accelerator [MATER] Substance that speeds chemical fermentation (as for wines) without participating in the resulting chemical changes; can be an enzyme or other catalytic agent. { ,fər·mən'tā·shən ak'sel·ə,rād·ər }

fermentation tube [MICROBIO] A culture tube with a vertical closed arm to collect gas formed in a broth culture by microorganisms. { ,fər·mən'tā·shən ,tüb }

fermenter [FOOD ENG] A vessel used for fermenting, such as a vat for fermenting mash in brewing. { fər'ment·ər }

ferment oil [MATER] A volatile oil formed by the fermentation of plant material in which the oil was not present originally. { ¦fər¦ment ,ȯil }

fermi *See* femtometer. { 'fer·mē }

Fermi age [NUCLEO] The value calculated for the slowing-down area in the Fermi age model; it has the dimensions of area, not time. Also known as age; neutron age; symbolic age of neutrons. { 'fer·mē ,āj }

Fermi age equation [NUCLEO] An equation in the Fermi age model which states that the Laplacian of the slowing-down density equals the partial derivative of the slowing-down density with respect to the Fermi age. { 'fer·mē ¦āj i,kwā·zhən }

Fermi age model [NUCLEO] A model used in studying the slowing down of neutrons by elastic collisions; it is assumed that the slowing down takes place by a very large number of very small energy changes. { 'fer·mē ,āj ,mäd·əl }

Fermi beta-decay theory [NUC PHYS] Theory in which a nucleon source current interacts with an electron-neutrino field to produce beta decay, in a manner analogous to the interaction of an electric current with an electromagnetic field during the emission of a photon of electromagnetic radiation. { 'fer·mē ¦bād·ə di¦kā ,thē·ə·rē }

Fermi constant [NUC PHYS] A universal constant, introduced in beta-disintegration theory, that expresses the strength of the interaction between the transforming nucleon and the electron-neutrino field. { 'fer·mē ,kän·stənt }

Fermi derivative [RELAT] A generalization of covariant differentiation along a curve that reduces to covariant differentiation when the curve is geodesic; an orthonormal tetrad constructed at each point along a timelike curve such that the Fermi derivative of the tetrad along the curve is zero has (1) its timelike basis vector equal to the curve's unit tangent vector and (2) its spatial basis vectors nonrotating along the curve. { 'fer·mē də,riv·əd·iv }

Fermi-Dirac distribution function [STAT MECH] A function specifying the probability that a member of an assembly of independent fermions, such as electrons in a semiconductor or metal, will occupy a certain energy state when thermal equilibrium exists. { ¦fer·mē di¦rak ,dis·trə'byü·shən ,fəŋk·shən }

Fermi-Dirac gas *See* Fermi gas. { ¦fer·mē di¦rak ,gas }

Fermi-Dirac statistics [STAT MECH] The statistics of an assembly of identical half-integer spin particles; such particles have wave functions antisymmetrical with respect to particle interchange and satisfy the Pauli exclusion principle. { ¦fer·mē di¦rak stə'tis·tiks }

Fermi distribution [SOLID STATE] Distribution of energies of electrons in a semiconductor or metal as given by the Fermi-Dirac distribution function; nearly all energy levels below the Fermi level are filled, and nearly all above this level are empty. { 'fer·mē ,dis·trə,byü·shən }

Fermi energy [STAT MECH] **1.** The average energy of electrons in a metal, equal to three-fifths of the Fermi level. **2.** *See* Fermi level. { 'fer·mē ,en·ər·jē }

Fermi gas [STAT MECH] An assembly of independent particles that obey Fermi-Dirac statistics, and therefore obey the Pauli exclusion principle; this concept is used in the free-electron theory of metals and in one model of the behavior of the nucleons in a nucleus. Also known as Fermi-Dirac gas. { 'fer·mē ,gas }

Fermi hole [SOLID STATE] A region surrounding an electron in a solid in which the energy band theory predicts that the

Transcript of Jeffrey John Chalmers, Ph.D.

Date: February 6, 2019
Case: Genentech, Inc., et al. -v- Amgen, Inc.

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IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF DELAWARE

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GENENTECH, INC., and :

CITY OF HOPE, :

Plaintiffs, : CA No. 17-1407-CFC

v. : (CONSOLIDATED)

AMGEN, INC., :

Defendant. :

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Videotaped Deposition of JEFFREY JOHN CHALMERS, PhD

Chicago, Illinois

Wednesday, February 6, 2019

8:36 a.m.

Job No.: 227140

Pages: 1 - 278

Reported by: Melanie L. Humphrey-Sonntag,

CSR, RDR, CRR, CRC, FAPR

Videotaped deposition of JEFFREY JOHN CHALMERS, PhD, held at the location of:

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Suite 3800
Chicago, Illinois 60602
(312) 962-3550

Pursuant to notice before Melanie L. Humphrey-Sonntag, a Certified Shorthand Reporter, Registered Diplomate Reporter, Certified Realtime Reporter, and a Notary Public in and for the State of Illinois.

"following fermentation" but -- yeah, you're asking me to ask what one word means. It's taken out of context. 08:54:45 08:54:46 08:54:49

Q So let's talk about the second term, "fermentation," and I would direct you to paragraph 48 of your declaration. 08:54:50 08:54:54 08:54:56

A Yes. 08:54:56

Q You state that, quote, "In the -- the ordinary meaning of 'fermentation' relates to activities such as making beer or wine, in which there is a chemical breakdown of a substance, e.g. sugar, usually under anaerobic conditions, by bacteria or yeast." 08:55:01 08:55:04 08:55:08 08:55:10 08:55:14 08:55:18

Did I read that correctly? 08:55:19

A Yes. 08:55:20

Q Is it your understanding that the Kao patent relates to a process for making beer or wine? 08:55:20 08:55:22 08:55:24

A It does not. 08:55:24

Q Then why did you believe that was the definition that was appropriate to apply in coming up with your opinion in this case? 08:55:25 08:55:28 08:55:30

MR. GUTMAN: Objection; mischaracterizes his declaration, mischaracterizes his testimony. 08:55:32 08:55:33

A The word "fermentation" is traditional, 08:55:38

referring to the metabolic -- not just this but if you want to start with -- let's start -- getting down further, it has "metabolic breakdown." It refers to the metabolism from an engineering biological point of view, so it's -- it's talking about that whole process. 08:55:44 08:55:48 08:55:50 08:55:52 08:55:56 08:56:02

And "fermentation" can be used -- to this day it's -- it's referred to in making biofuels. 08:56:04 08:56:07

THE COURT REPORTER: I'm sorry. "Making" -- 08:56:07 08:56:07

THE WITNESS: "Biofuels," alternative energy. 08:56:16 08:56:18

A (Continuing.) People will quite often use the word "fermentation," but even then they get a little iffy on what you're calling it. 08:56:18 08:56:19 08:56:24

So it is a firm and fast concept among our community, and it's considered sloppy to call -- to even use the term "cell" -- "fermentation" in the cell culture world. It's like you're -- "Why are you doing that? Call it 'cell culture.'" 08:56:26 08:56:30 08:56:34 08:56:37 08:56:41

Q In the context of making beer and wine, what is the process of fermentation, just briefly? 08:56:44 08:56:53

A I completely stand by what's here. But then to go the next step, which I think you're asking -- so I want to be on record that I agree 08:56:55 08:57:03 08:57:05

with what's here.
And then go the step further, in the traditional sense, it's in an anaerobic environment. The sugars are broken down; the organism gets its energy from that. The waste products, instead of us, as humans, making -- just using -- taking oxygen and making CO2, the organisms are dumping electrons onto molecules that then become ethanol, lactic acid, acetic acid, all array of different compounds.
When we go out and exercise, don't get enough oxygen in our body and our muscles ache, that's anaerobic fermentation going on in our body. That is -- they're making lactic acid.
Q So if I could simplify, it's the process of the cells taking sugars and producing other chemicals with them?
MR. GUTMAN: Objection; mischaracterizes his testimony, asked and answered.
A Not other specific chemicals, very specific chemicals.
Q So it's a process of cells breaking down sugars and producing specific chemicals?
A That's right.
Q Okay.

A Very specific. And the reason I say that, it's the dream of many of my colleagues to be able to do it better because we could solve our energy problems. 08:58:15 08:58:17 08:58:21 08:58:24

So there's very specific chemicals these organisms make, and we'd like them to be certain kinds of fuels. So it's not a random thing at all. It's very specific. 08:58:26 08:58:29 08:58:33 08:58:35

Q And while the cells are producing these specific chemicals, are they growing? 08:58:38 08:58:40

A Yes, almost always. 08:58:43

There could be a few exceptions, but it's very much considered classified in my community as a growth-associated product. 08:58:45 08:58:47 08:58:50

Q So in -- in the context of "fermentation" as discussed in paragraph 48 of your declaration, cells are both growing and producing; is that right? 08:58:54 08:58:56 08:59:00 08:59:02

A That is correct. 08:59:03

MR. GUTMAN: Objection; vague, lacks foundation. 08:59:04 08:59:07

Q Does the term "fermentation" have any meaning in the context of antibody production? 08:59:09 08:59:11

A In a sloppy manner. It should be saying "cell culture" because, bringing this back, you're 08:59:14 08:59:20

growing the cells -- these are genetically engineered cells to produce a specific product. That engineering is what makes the antibody. 08:59:29 08:59:31 08:59:33

Normal cells that make beer and wine or make yeast don't make antibodies. 08:59:38 08:59:41

Q So I think I've understood your testimony to be that the term "fermentation" does have a meaning in the context of antibody production but it's a sloppy meaning. Is that right? 08:59:46 08:59:48 08:59:50 08:59:52

MR. GUTMAN: Objection; mischaracterizes his testimony, asked and answered. 08:59:54 08:59:55

A If you get into the very -- again, the fundamental definition of "fermentation" is as I wrote in here. It is the process anaerobically by which sugars are converted to ethanol, lactic acid, acetic acid, butanol, and it's a process by which energy is generated by the organisms. 08:59:57 09:00:01 09:00:05 09:00:09 09:00:16 09:00:20

In an antibody production of CHO cells, they are not getting any energy by making antibodies, none, zero. In fact, that's a drain of energy. So it's a fundamentally different metabolic process. Fundamentally different, not comparable at all. 09:00:24 09:00:28 09:00:30 09:00:33 09:00:35 09:00:38

Q And let me ask my question a different way. 09:00:39 09:00:41

If you could look -- I believe it's the second sentence of paragraph 48 of your declaration. You say, "'Fermentation' is not typically used to refer to culturing mammalian cells." 09:00:41 09:00:44 09:00:46 09:00:49 09:00:52

Do you see that? 09:00:53

A Yes. 09:00:54

Q I want to ask you a couple questions about that statement. 09:00:56 09:00:59

Is it your understanding that if a term has multiple meanings, the person of ordinary skill has to determine which one is most common to figure out the ordinary meaning? 09:01:00 09:01:01 09:01:04 09:01:07

MR. GUTMAN: Objection; calls for a legal conclusion, foundation. 09:01:08 09:01:10

A As someone skilled in the art, we're immediately going to go, "Why is that -- why are they using the word 'fermentation'"? 09:01:13 09:01:21 09:01:24

If read the patent -- which I did, as we discussed at the beginning -- my first question was, "Why are they saying 'fermentation'? Why are they saying 'fermentation' in the claim?" Doesn't make sense to me. It adds indefiniteness, as I keep coming back to that concept. It made it indefinite to me. 09:01:27 09:01:29 09:01:32 09:01:35 09:01:39 09:01:42 09:01:46

But if you push me hard enough, I'm going to have to say, "All right. They have -- they have not defined it in the patent, but they do have a clear statement saying, 'Following fermentation proteins are purified.'" 09:01:47 09:01:49 09:01:51 09:01:54 09:01:56

So now if I also go back to the prosecution history -- in fact, you could make the argument that at least one of the papers that the examiner pulled was definitely much more in the tradition of fermentation. And for Genentech to get their claim, they had to associate fermentation with cell growth and the production which was going on in those vessels. And so Genentech then said "Okay. We're going to use the word 'following fermentation' to -- to construct our claim around." And that meant after cell growth. 09:02:00 09:02:05 09:02:10 09:02:13 09:02:18 09:02:25 09:02:27 09:02:32 09:02:36 09:02:39 09:02:41 09:02:44

So you just asked me a minute ago "fermentation." That right there -- if I look at the whole prosecution history, they're locking fermentation into this more traditional view of a metabolic process of taking a sugar or some compound and breaking down, getting energy from. 09:02:44 09:02:48 09:02:53 09:02:54 09:02:57 09:03:00

CHO cells that are used -- and in Claim 10 of the patent we've got potential prokaryotes. 09:03:06 09:03:10

It's not -- the patent is not just about animal cells. Those prokaryote cells, when they make antibody, they are using energy from sugar, kicking off CO2. They're not making ethanol. They're -- you do not want ethanol when you're doing those kind of cultures. You need oxygen. 09:03:14 09:03:17 09:03:20 09:03:24 09:03:27 09:03:28

So, again, when I read it, I go, "Okay. The fermentation here sounds like they're trying to link it to the cell growth, that production, but definitely not making -- they're -- they're definitely not making ethanol. They're not making a product where they're dumping their electrons onto it." 09:03:31 09:03:34 09:03:38 09:03:43 09:03:45 09:03:47 09:03:49

Q Thank you. 09:03:51

I noted in the sentence we just read that you use the word "typically." You said "Fermentation is not typically used to refer to culturing mammalian cells"? 09:03:51 09:03:53 09:03:54 09:03:58

A Yeah. 09:03:58

Q So I take it that it's fair to say that "fermentation" sometimes is used to refer to culture in mammalian cells. 09:03:59 09:04:02 09:04:03

MR. GUTMAN: Objection. Is -- is that a question or -- 09:04:04 09:04:06

Q Is that correct? 09:04:07

MR. GUTMAN: -- or testimony? 09:04:07

A Sometimes. Sometimes it's used. 09:04:08

That's where I -- when I'm using the word 09:04:14

"sloppy" -- again, my community is not using -- 09:04:16

words are precise to us but not in the same legal 09:04:23

sense. And I'm not a lawyer. 09:04:26

But we turn our nose up at that. We would 09:04:31

say, "That's sloppy. You shouldn't say that 09:04:34

because it's misleading. It leads you somewhere 09:04:37

else." 09:04:39

I have a PhD student currently who -- who 09:04:40

is working with a contract manufacturing 09:04:42

organization that makes -- does biosimilar cells 09:04:46

and other things. I was recently there and I -- 09:04:51

they showed me their process, and I asked them, 09:04:54

"Do you use the word 'fermentation'?" And he just 09:04:58

turned his nose up, just as I'm saying. No, we 09:05:02

would not call it that. It's a cell culture 09:05:05

process. 09:05:07

Q What are some of the times that 09:05:07

"fermentation" is used to refer to culturing 09:05:10

mammalian cells? 09:05:12

A Obviously, this patent, '69, does. That's 09:05:13

why we're talking about it. 09:05:17

Q Other than this patent have you ever heard 09:05:18

the term "fermentation" used for -- for culturing mammalian cells? 09:05:19 09:05:23

A Very rarely. 09:05:25

And the Cell Culture Engineering Award I talk about is, which is highlighted -- 4. 09:05:27 09:05:32

Why I keep highlighting that -- excuse me for my voice cracking -- it is -- this is -- this meeting has been ongoing for 30 years. There's really two meetings, that meeting and a European meeting, where all of us -- most of us that work in this field that do what I am talking about here, we come every two years, and we discuss all -- so much of this. 09:05:37 09:05:39 09:05:44 09:05:47 09:05:55 09:05:57 09:06:00 09:06:03

No one -- I mean, has it happened? Yeah. In 30 years, every two years, I'm sure there's been some. But Cell Culture Engineering -- that's the name of the meeting. We speak of it as cell culture. We don't have sessions on fermentation of CHO cells, no. You wouldn't do it. 09:06:05 09:06:10 09:06:13 09:06:16 09:06:20 09:06:22

So that's why I say "typically." I'd probably say it's rarely done. I'm sure if you looked hard, you could find examples. But it's not typical. 09:06:24 09:06:26 09:06:28 09:06:34

Q But you have never personally used the term "fermentation" to refer to culturing 09:06:34 09:06:37

mammalian cells? 09:06:41

A I might have been sloppy once myself. But 09:06:41

the vast majority of the time I haven't. Yeah, 09:06:43

you can probably find somewhere in 30 years that 09:06:46

I've used that word. Maybe. 09:06:48

Q The Cell Culture Engineering conference 09:06:49

you talked about earlier, are you involved in 09:06:52

running that conference in any respects? 09:06:56

A Yes. 09:06:58

Q How so? 09:06:58

A I chaired the meeting twice in the '90s -- 09:06:59

well, cochaired it -- and I cochaired it 09:07:03

four years ago. 09:07:07

Q What were your responsibilities as chair 09:07:07

of the meeting? 09:07:12

A Picking the session chairs, picking the 09:07:13

location. 09:07:26

We have an advisory board. It's approving 09:07:28

that, in a sense, because, you know, there's a 09:07:32

tradition of how that continues. So we -- we 09:07:34

agree with -- pick the leadership team of the 09:07:40

meeting itself who's overseeing the meeting, like 09:07:42

I said, the location, the price, how much we're 09:07:45

charging. It gets down into the weeds as much as 09:07:46

even menus. 09:07:49

But more -- more globally here with our scientific advisory panel, we'll figure out what's the focus of the meeting, what are the important topics. I think this is really important. It's made up of half academics, half industrialists, and we make a real goal of being in touch with what the issues are. 09:07:50 09:07:55 09:08:00 09:08:02 09:08:04 09:08:08 09:08:13

Q Is this a prestigious conference? 09:08:14

A Yes. 09:08:19

Q Are papers presented at this conference? 09:08:20

A Pretty much so. 09:08:22

Q And I imagine it's hard to get your paper presented at the cell culture conference. 09:08:25 09:08:28

A Very hard. 09:08:30

Q What is the screening process like for papers presented at the cell culture conference? 09:08:31 09:08:33

A Well, to answer your question -- let me -- let's go back for a second. 09:08:36 09:08:38

As of now it's very hard. If you go back into the earlier days, the late '80s, not as hard. Because in the late '80s, it was a dream to see cell culture be the way it is today. The -- the market size was not anticipated. 09:08:40 09:08:43 09:08:49 09:08:58 09:09:00

As the market grew, got bigger and bigger, then it became really prestigious and hard to get 09:09:03 09:09:10

a paper accepted. 09:09:13

So there's a progression. I want to get 09:09:14

that straight. 09:09:16

Q Okay. 09:09:16

A What other -- okay. 09:09:20

And -- so I probably didn't answer your 09:09:22

question. Go back, please. I wanted to back up 09:09:24

for a minute so -- 09:09:26

Q Yeah, I don't think -- I -- I don't think 09:09:27

you did. 09:09:28

A So -- 09:09:28

Q My question was, what is the process for 09:09:29

getting a paper selected at the Cell Culture 09:09:30

Engineering conference? 09:09:34

A As I mentioned, as a chair, as chair of 09:09:35

the meeting, we pick our cochairs. 09:09:38

We, in general, do not -- and I didn't 09:09:41

micromanage it -- so the actual picking of the 09:09:48

papers are the responsibility of the cochairs -- 09:09:50

I mean, the -- sorry -- the session chairs. I've 09:09:55

got to make sure I get the word right. The -- the 09:09:57

session chairs. 09:10:00

So we have -- I'm -- we have cochairs 09:10:01

that oversee it, then we'll have eight, 09:10:04

nine different sessions. We pick the chairs of 09:10:07

the sessions, and those are usually an industrial and an academic. 09:10:13 09:10:16

They -- we then have a call for papers. They bring in people or they invite people as well as we have a call for papers. 09:10:17 09:10:20 09:10:27

They sit down -- "they" being the session chairs -- they decide. Very rare does a meeting chairperson override the choices of the session chairs. That would be a -- it might happen, but that's basically implying you didn't do your job of picking the proper session chairs. 09:10:29 09:10:31 09:10:36 09:10:42 09:10:48 09:10:49

So, you know, it's possible there could be some sloppiness at that point even. 09:10:52 09:10:57

In terms of terms being used, would we override it on that? Probably not unless it was really bad. 09:11:04 09:11:07 09:11:10

And, you know, we basically, after the meeting, get our buddies together -- people we trust -- and we ask, "Okay. Who did a good job running the session? Papers good? Not so good?" 09:11:12 09:11:14 09:11:17 09:11:21

I was just in a meeting last week, did -- we exactly did that. We got aside and said, "Yeah, they shouldn't have invited these people. That was not good." 09:11:26 09:11:29 09:11:32 09:11:35

Q And when you decide that a paper is good, 09:11:36

is there a collection or anything done after the conference of the best papers? 09:11:38 09:11:40

A Yes. Not always. It's less -- it's less common now. 09:11:41 09:11:48

So in the early days there was. However, the journal -- so they would be collected -- we call them proceedings. 09:11:52 09:11:56 09:11:59

And as the years have gone by -- now -- again, now I'm speaking purely as an academic here, which you're expecting of me, but I want to preface that that -- there's been a fundamental change over the years that now people are really worried -- researchers are much more worried about the prestige of the journal. 09:12:01 09:12:05 09:12:08 09:12:11 09:12:13 09:12:16 09:12:18

So nowadays, the most recent decade, the journals -- the collection -- a lot of people elect not to do it, elect not to put a paper that they presented into these journals that are proceedings because these were not considered prestigious. 09:12:20 09:12:26 09:12:29 09:12:32 09:12:36 09:12:37

Q I see. But at the time that you were cochairing the meeting in the '90s, it was still prestigious to have your paper selected for inclusion? 09:12:38 09:12:40 09:12:43 09:12:44

A To be selected for presentation is 09:12:45

prestigious. Even then you'd opt out. If you had a really great paper, you wouldn't put it in the proceedings. You would try to go for a great name journal, which typically has better peer review. 09:12:49 09:12:52 09:12:54 09:12:59

But that's -- we can go down the road of peer review, but that's a different -- I don't think we're here to discuss the merits of peer review. 09:13:02 09:13:05 09:13:08 09:13:09

Q No. I'm sure you could talk all day about the merits of peer review. 09:13:10 09:13:13

A That's exactly right. 09:13:14

Q I'm -- I'm going to hand you what's going to be marked as Exhibit 63. 09:13:16 09:13:18

(Deposition Exhibit 63 marked for identification and attached to the transcript.) 09:13:30 09:13:32

MR. GUTMAN: Thank you. 09:13:32

MR. MC CLOUD: You're welcome. 09:13:32

Do you guys want one? 09:13:34

MS. GETTEL: Thanks. 09:13:36

A Yep, I remember this meeting. 09:13:37

This was one of the ones I hosted. You're right. 09:13:40 09:13:42

BY MR. MC CLOUD: 09:13:42

Q So just so the record is clear, this is an exhibit titled "Cell Culture Engineering VI," and 09:13:45 09:13:48

I take it from the statement you just made this is the proceedings of the conference that you cochaired. Is that right? 09:13:51 09:13:53 09:13:57

A Yes. 09:13:58

Q And I see your name is listed on the cover here. What role did you have in putting together this publication? 09:13:58 09:14:06 09:14:08

A Let me make sure. 09:14:10

This was one of the two meetings that I was cochairing with Rob Arathoon, who was -- I forget his title. He was at Genentech. I don't -- you know, I'd have to go back and check his title. 09:14:14 09:14:20 09:14:29 09:14:32 09:14:34

Q Okay. It says that you were one of the editors of this publication on the cover page. Is that correct? 09:14:35 09:14:37 09:14:39

A Technically Mike Betenbaugh was. We delegated it to Mike Betenbaugh and Alison. So it's the -- how do I want call it? 09:14:39 09:14:44 09:14:49

Protocol, the etiquette at the time would list Rob and I because we ran the meeting. And then these -- the people that were the editors -- as I mentioned before, we delegated it. So we would delegate the authority to that, to the chairs. 09:14:53 09:14:55 09:14:58 09:15:01 09:15:03 09:15:07

Because to be presented here, you had to be a -- to get a -- these are representative of some of the papers that were presented, not all. 09:15:08 09:15:10 09:15:14

These were -- these had -- to be in here, they had to be picked by one of the cochairs -- I mean -- cosession leaders. I keep misspeaking here. 09:15:17 09:15:22 09:15:25 09:15:28

So there's a co- -- there's a chair and cochair on top, then we have session chairs. The session chairs pick the papers that are presented. The papers that are presented have the option to -- to be published in this and get peer reviewed. 09:15:29 09:15:31 09:15:34 09:15:40 09:15:43 09:15:46

And this journal is an example of what I said, that people don't let -- this is not considered that prestigious of a journal anymore so nowadays people don't. 09:15:47 09:15:49 09:15:53 09:15:56

Q So the papers in this publication were selected by the session chairs -- 09:15:58 09:16:01

A That's correct. 09:16:03

Q -- at the conference? 09:16:04

A That's correct. 09:16:05

Q Can you turn to page 19? 09:16:05

A Yes. 09:16:13

Q Are you there? 09:16:20

A Yes. 09:16:20
Q Okay. And you see there's an article 09:16:22
entitled "Effects on Growth Behavior in Continuous 09:16:23
Hybridoma Cell Cultures: The Role of Viral 09:16:26
Contamination"? 09:16:30
Do you see that title? 09:16:31
A "Hybridoma Cell Cultures, the Role of 09:16:32
Viral Contamination," that's correct. 09:16:41
Q What is a hybridoma? 09:16:42
A Hybridoma is a fusion of an antibody- 09:16:45
producing cell with a type of cancer cell that 09:16:52
allows them to be immortalized. 09:16:55
Q And could you turn to page 20, please. 09:16:57
Are you on page 20? 09:17:09
A I am. 09:17:10
Q And you see there's a section entitled 09:17:11
"Materials and Methods"? 09:17:13
Do you see that? 09:17:14
A Yes. 09:17:15
Q And under "Materials and Methods" there's 09:17:15
a subheading for "Cell Cultures"? Do you see 09:17:18
that? 09:17:21
A Yes. 09:17:21
Q Okay. Could you read the first sentence 09:17:22
under the subheading "Cell Cultures" out loud, 09:17:25

please? 09:17:29
A "The cell line used in the present study 09:17:29
was the rat-mouse hybridoma cultured in a 09:17:33
serum-free medium based on DMEM/F12" -- keep 09:17:38
reading? -- "with human transferrin and bovine 09:17:43
insulin. The amino acids were supplemented to 09:17:50
necessary concentrations." 09:17:52
Q Thank you. 09:17:53
A rat-mouse hybridoma, that is a mammalian 09:17:54
cell; is that correct? 09:17:58
A Correct. 09:17:59
Q And I saw a reference there to bovine 09:18:00
insulin. What is insulin? 09:18:03
A A growth hormone -- well, it's not just a 09:18:04
growth hormone. It is a hormone that is involved 09:18:07
in uptake and regulation of sugar, among other 09:18:12
things, in animal cells. 09:18:17
Q And it said "bovine insulin." Is that cow 09:18:18
insulin? 09:18:22
A Correct. 09:18:23
Q Are there different kinds of insulin? 09:18:24
A There is. 09:18:27
Q So if you look further down the page, 09:18:28
you'll see another subheading that says 09:18:30
"Fermentation Process." 09:18:32

Do you see that? 09:18:33
A Yes, I do. 09:18:34
Q And the first sentence under fermentation 09:18:34
process says, "After inoculation the perfusion 09:18:36
fermentation process, Figure 2, was started with a 09:18:38
dilution rate of D equals 1.75D to the minus 1." 09:18:40
Do you see that? 09:18:46
A Yes, I do. 09:18:47
Q Is the fermentation process that's being 09:18:48
referred there a process for making beer and wine? 09:18:50
A It is not. 09:18:52
Q It's a process for making -- for culturing 09:18:53
mammalian cells; correct? 09:18:59
A That is what's being described here. And 09:19:01
it's also very sloppy. And the reviewers should 09:19:03
have clipped that. 09:19:06
I did not deny earlier that those terms 09:19:12
get in. This is why it's sloppy. 09:19:14
Q Do you recall anyone, when this article 09:19:20
was being reviewed, commenting that the use of 09:19:23
"fermentation" here was incorrect? 09:19:27
A I was not one of the reviewers of this 09:19:28
article. I can honestly say that. 09:19:32
Q Do you recall anyone commenting, when this 09:19:35
article was presented at the conference, that they 09:19:41

didn't understand what "fermentation" meant in this context? 09:19:44 09:19:46

A This would be an example of a lot of us shaking our head, going, "They shouldn't be saying that." 09:19:47 09:19:50 09:19:54

Q Would someone in your field understand what authors of this paper were referring to when they said "fermentation process"? 09:19:55 09:19:58 09:20:00

A As it's shown here, yeah. And it's also very poor because you're still going, "All right. What point is metabo- -- metabolism going on and when is product being made?" 09:20:02 09:20:07 09:20:11 09:20:18

It causes a vagueness. 09:20:20

So it's sloppy, imprecise. 09:20:23

Q You can put that to the side. 09:20:37

I'll hand you what is going to be marked as Exhibit 64. 09:20:39 09:20:41

(Deposition Exhibit 64 marked for identification and attached to the transcript.) 09:20:49 09:20:51

Q Do you recognize Exhibit 64? 09:20:51

A I need -- I would need to spend some time looking this over. 09:21:06 09:21:09

I know Sadettin Ozturk, and I know he has edited a number of collections like this. In fact, I've been involved in some of his 09:21:14 09:21:18 09:21:20

collections.
Whether -- I need to figure out which one this is and when it was, so you need to give me time to look it over.
Q Sure. I think if you turn to the contents table, page 6, you'll see that you were involved in this collection.
A Yeah. That's what I say, I could have been.
Q Okay.
A But there's -- he's done some that I haven't and some that I have so --
Q And you said that you know Dr. Ozturk; is that right?
A Yeah.
Q What is his reputation in the field?
A Well respected.
Q Who is the audience for the -- a book like this?
MR. GUTMAN: Objection; lacks foundation, vague.
A Actually, a way to find out who the audience is is probably very -- for me to sit down and read the preface. That's usually where you list that.

So -- I mean, I could spend some time now if you want me to read it. 09:22:28 09:22:31

Q Please go ahead. 09:22:32

A But -- okay. 09:22:33

It doesn't really say at that point what they're addressing it to in general, so I wasn't really correct in saying it usually does. 09:23:51 09:23:52 09:23:54

But it does sort of cover what's going on, which it should. Yes. Okay. 09:23:56 09:23:59

Q So what is going on with this book? 09:24:01

A I -- 09:24:02

MR. GUTMAN: Objection; vague, calls for a narrative. 09:24:02 09:24:06

MR. MC CLOUD: Let me withdraw that. I'll ask a different question. 09:24:10 09:24:11

Q You submitted a paper for inclusion in this book; is that right? 09:24:12 09:24:15

A Yeah. Let me see which one it is. 09:24:15

Yep, down here, 225. Yep. 09:24:18

I did. Chapter 7. Or -- I think it's a chapter -- yep. 09:24:20 09:24:25

Q And this was not a chapter that you submitted for the general population; right? 09:24:27 09:24:29

A No. 09:24:31

Q This is for -- 09:24:35

A Well, what -- what do you mean by "general population"? 09:24:36 09:24:37

Q The average person on the street. This is a chapter that you wrote for your colleagues in the industry; right? 09:24:38 09:24:39 09:24:42

A That would be a better description than "a person on the street." That would be better. 09:24:44 09:24:46

Q Okay. So this is a resource for individuals in industry who want to learn about new topics in cell culture technology; is that right? 09:24:48 09:24:51 09:24:53 09:24:55

A And summarize, correct. 09:24:55

Q Okay. Could you turn to Chapter 15 starting on page 523? 09:24:57 09:25:01

A Chapter 15? 09:25:05

525? 09:25:11

Q 523. 09:25:12

A 523. 09:25:13

Yes. 09:25:28

Q Okay. And you see there's a chapter entitled "Validation of Cell Culture-Based Processes and Qualification of Associated Equipment and Facility"? 09:25:28 09:25:30 09:25:32 09:25:34

Do you see that? 09:25:36

A Correct. 09:25:36

Q And this chapter's authored by a Chandra M. Dwivedi. Do you know Mr. Dwivedi? 09:25:37 09:25:40

A No. I'm not familiar with the name. 09:25:43

Q Okay. And if you look, there is an asterisk after his name. Do you see that? 09:25:45 09:25:48

A Yes. 09:25:51

Q If you look down at the bottom of page 523, it says Mr. Dwivedi is currently at Biogen Idec, Inc., Cambridge, Massachusetts, USA. 09:25:51 09:25:54 09:25:58

A Correct. 09:25:58

Q Do you see that? 09:26:02

A Yes. 09:26:02

Q Do you know what Biogen Idec is? 09:26:03

A Yes, I do. 09:26:06

Q What is Biogen Idec? 09:26:07

A A biotech -- biotechnology company. 09:26:11

Q And they make therapeutic antibodies? 09:26:13

A They do. 09:26:15

MR. GUTMAN: Objection; lacks foundation. 09:26:16

Q Do they make their therapeutic antibodies using mammalian cells? 09:26:17 09:26:21

MR. GUTMAN: Lacks foundation, vague. 09:26:21

A I believe so. 09:26:23

Q Could you turn to page 529, please? 09:26:24

A Yes. 09:26:26

Q Okay. And you see there's a section titled "Cell Culture and Fermentation Process"? 09:26:34 09:26:36

Do you see that? 09:26:39

A I do. 09:26:40

Q Okay. And the third sentence of that paragraph states, "Commercial fermentation processes and bioreactor technologies have been developed in the last several decades to state-of-the-art production of pharmaceutical agents of interest." 09:26:41 09:26:46 09:26:48 09:26:52 09:26:53 09:26:56

Do you see that? 09:26:57

A Yeah. Took me a minute. You were a little fast there. But -- so I -- I caught on, though. I got it, yeah. 09:26:57 09:27:01 09:27:04

Q Okay. And is it your understanding that the fermentation processes being referred to in that sentence are processes for making beer and wine? 09:27:05 09:27:07 09:27:09 09:27:11

A It is a sloppy use of the word, and I'm consistent with what I said earlier. They pushed -- "You will, by association, say it's cell culture" -- in this case but it's sloppy. It should not have been used. 09:27:12 09:27:17 09:27:20 09:27:24 09:27:27

Q But you understand that the process of fermentation that's being referred here -- to here 09:27:29 09:27:30

is a process for making therapeutic antibodies? 09:27:33
MR. GUTMAN: Objection; lacks foundation, 09:27:36
calls for speculation. 09:27:39
A By inference, that's what they're 09:27:40
referring to. I'm capable of inferring it. But 09:27:42
as we have discussed previously, if I go look up 09:27:46
the word -- and as I understand it fully well -- 09:27:50
it is sloppy. It should have been edited out. 09:27:57
Someone shouldn't have -- should have edited that. 09:28:01
I did not edit that chapter. 09:28:02
And as I said, it could -- it can slip 09:28:06
through. You might be able to find it in my work, 09:28:08
but it will slip through. It's -- it's sloppy. 09:28:11
Q Can you turn to page 531, please? 09:28:14
A Yes. 09:28:18
Q Okay. And you'll see at the bottom of the 09:28:20
page it says "Production Scale Fermentation." 09:28:21
Do you see that? 09:28:24
A Yes. 09:28:24
Q And the first sentence after that heading 09:28:25
says, "Fermentation at the production scale may be 09:28:29
carried out in a vessel (fermentor or bioreactor), 09:28:32
bottle, or a bag depending on the product type." 09:28:40
Do you see that? 09:28:43
A Yes, I do. 09:28:43

Q Is it your understanding the production scale fermentation being referred to in that sentence is a process for making beer and wine? 09:28:43 09:28:46 09:28:48

A It is my -- my answer has not changed. It's sloppy. 09:28:50 09:28:52

Q I'm going to ask my question again. 09:28:53

Is it your understanding that the production scale fermentation being referred to in that sentence is a process for making beer and wine? 09:28:57 09:28:58 09:29:01 09:29:03

MR. GUTMAN: Objection; lacks foundation. 09:29:03

A And I'm saying that that is a sloppy, imprecise use of the word and should have been edited out. 09:29:05 09:29:09 09:29:11

Q You understand that the fermentation process being referred to in that sentence is a process for making a therapeutic antibody; correct? 09:29:13 09:29:15 09:29:17 09:29:24

MR. GUTMAN: Objection; lacks foundation, calls for speculation. 09:29:24 09:29:26

A I know that Biogen Idec does not make beer and wine. So by inference -- I have to infer that they mean "of." They should have said -- they actually didn't say "bio." Why they used "fermentor" -- there -- there's an inference there 09:29:28 09:29:33 09:29:37 09:29:39 09:29:41

and it's sloppy. It shouldn't have been used. 09:29:44

Q I wanted to ask you about the term 09:29:46

"fermentor." What is a fermentor? 09:29:48

A Historically a fermentor was what they 09:29:51

made moonshine in. 09:29:57

Then, as the field became more commercial, 09:30:00

people started using -- like, for instance, 09:30:05

decades ago New Brunswick Scientific made 09:30:09

fermentors for bio- -- for not just 09:30:13

fermentation but bioenergies. 09:30:18

In the 1970s they were doing it, and then 09:30:19

they would make it for bacterial work. And then 09:30:22

it started getting a little back and forth -- 09:30:24

"Well, we should start calling -- stop calling 09:30:26

them fermentors and calling them bioreactors." 09:30:28

So there was an evolution because these 09:30:31

vessels went from just doing -- making beer and 09:30:34

wine and traditional and -- traditional metabolic 09:30:36

by-products to genetic engineering, which started 09:30:39

in the late '70s, early '80s. 09:30:42

And people then began to start saying, "We 09:30:45

should call them bioreactors because we're not 09:30:47

fermenting it now. We are doing -- even bacterial 09:30:50

work is making a recombinant protein." So there 09:30:53

began to be a division. 09:30:57

And we want to call that fermentors that does fermentation and it -- bioreactors here. So this is actually kind of representing that breaking apart. 09:30:58 09:31:00 09:31:03 09:31:05

And they should have did -- like I said, that shouldn't have been used but it is. We just kind of go, "Oh, well, being sloppy again." So it's by inference. 09:31:06 09:31:08 09:31:11 09:31:17

Q And, again, you're inferring, based on the context, that this is referring to fermentation being a process for making antibodies? 09:31:19 09:31:21 09:31:24

A That's right. 09:31:27

MR. GUTMAN: Objection; mischaracterizes his testimony, lacks foundation. 09:31:28 09:31:29

A So bring -- it's bringing us back to the claim. And you asked me -- let me read my -- No. 47 here. 09:31:33 09:31:36 09:31:40

"I also understand that Amgen has proposed that 'following fermentation' is indefinite, which I am informed means that the scope of the claim could [sic] not be reasonably certain to a person skilled in the art, such that it would not be reasonably certain to a person skilled in the art when fermentation ends and steps following fermentation begin. I agree that 'following 09:31:43 09:31:45 09:31:48 09:31:52 09:31:55 09:31:56 09:31:58 09:32:02

fermentation' is indefinite. However, if a person skilled in the art were to seek an interpretation of this term despite its indefiniteness, a person skilled in the art would find Amgen's construction to best reflect the limited guidance given in the patent." 09:32:05 09:32:08 09:32:12 09:32:16 09:32:17 09:32:21

So when I read this statement you're saying, it's the exact same kind of thinking -- or at least a very similar one. I shouldn't be so exact. It's very similar. I'm inferring it. 09:32:21 09:32:23 09:32:26 09:32:29

I'm having to interpret it and go with -- and they're helping me here by saying "fermentor or bioreactor." So in this context they're trying to equate it. 09:32:33 09:32:36 09:32:39 09:32:43

I -- you didn't ask me but I was deposed eight years ago on an import/export dispute from a -- a biotech company that makes bioreactors. They were explicitly clear they make bioreactors. They don't make fermentors. 09:32:45 09:32:47 09:32:54 09:32:59 09:33:04

And it is the same reactor that's used in the '869 patent. It's a 2-liter Applikon mentioned in the '869 patent. That is a -- that is an Applikon bioreactor. I went back and even looked. They never called it a fermentor. They were very clear of calling it a bioreactor because 09:33:07 09:33:11 09:33:17 09:33:21 09:33:25 09:33:27

that would be looked down upon in selling a product. "We don't sell fermentors. We sell bioreactors to the biotech industry." 09:33:30 09:33:32 09:33:35

Q Are you finished? 09:33:38

A (No verbal response.) 09:33:38

MR. MC CLOUD: I'll move to strike that answer as nonresponsive, and I'll ask my question again. 09:33:42 09:33:43 09:33:44

Q So you understand, based on the context of this sentence, that the use of the term "fermentation" is referring to a process for making antibodies? 09:33:45 09:33:47 09:33:50 09:33:51

MR. GUTMAN: Objection; asked and answered, lacks foundation. 09:33:53 09:33:55

A Because I'm skilled in the art and I have to make an interpretation despite it being indefinite, I am saying that this statement, which I keep saying is correct -- I stand by it. 09:33:59 09:34:03 09:34:06 09:34:10

Q I'm sorry. Which statement are you referring to? The statement in your declaration? 09:34:13 09:34:15

A Correct. 09:34:18

Q I'm asking about the sentence that we just read in the Dwivedi chapter. So I'll ask my question again for the third time. 09:34:19 09:34:21 09:34:25

You understand, based on the context of 09:34:26

this sentence, that they are referring to a fermentation process for the process of making antibodies? 09:34:29 09:34:30 09:34:33

MR. GUTMAN: Objection; asked and answered, lacks foundation, vague. 09:34:34 09:34:35

A I have to infer it because the word "fermentor" immediately sends up a flag on it. Why are they mentioning that? That's sloppy. 09:34:38 09:34:42 09:34:47

So in the whole context of the article from what -- I can't -- I might have read this a long time ago. But as I look it over, they're constantly referring to making a product that's a drug. 09:34:50 09:34:52 09:34:54 09:34:58 09:35:01

So that would imply that, yes, you're correct; it's making a human drug and not making beer or wine. So I'll -- I'll agree with that. 09:35:03 09:35:08 09:35:11

Q Thank you. 09:35:13

Could you turn to page 225, please. 09:35:20

Let me know when you get there. 09:35:27

A Yes. 09:35:29

Q Okay. And this is a chapter titled "Aeration, Mixing, and Hydrodynamics in Bioreactors." 09:35:34 09:35:37 09:35:40

A Yes. 09:35:40

Q Do you see that? 09:35:42

Amgen 2011 Annual Report and Financial Summary

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In many countries, the influence of regional and hospital payers also contributes to whether patients have access to certain products. For example, a product may be successfully listed on a national formulary, but may also be subject to further evaluations or competitive bidding by payers at a regional or hospital level. The impact of multiple layers of assessment can result in delay or failure to secure access and/or net price pressure.

Payers in some countries are using and others are beginning to experiment with alternative payment mechanisms (e.g., payment caps, risk sharing) as a means to maintain access to innovative therapies while increasing their budget certainty. Requirements for such payment mechanisms can impact Amgen's business through increased net price concessions and added administrative burden.

Fraud and Abuse Regulations Related to Reimbursement

As participants in government reimbursement programs, we are subject to various U.S. federal and state laws, as well as foreign laws, pertaining to healthcare "fraud and abuse," including anti-kickback laws and false claims laws. (See Government Regulation — Other.) Violations of fraud and abuse laws can result in stringent enforcement penalties up to and including complete exclusion from federal healthcare programs (including Medicare and Medicaid).

Manufacturing, Distribution and Raw Materials

Manufacturing

Biological products, which are produced in living systems, are inherently complex due to naturally-occurring molecular variations. Highly specialized knowledge and extensive process and product characterization are required to transform laboratory scale processes into reproducible commercial manufacturing processes. Our manufacturing operations consist of bulk manufacturing, formulation, fill and finish and distribution activities. Bulk manufacturing includes fermentation and/or cell culture, processes by which our proteins are produced, and also includes purification of the proteins to a high quality. The proteins are then formulated into a stable form. The fill process dispenses the formulated bulk protein into vials or syringes. Finally, in the finish process, our products are packaged for distribution.

We operate a number of commercial and/or clinical manufacturing facilities, and our primary facilities are located in the United States, Puerto Rico and the Netherlands. (See Item 2. Properties.) We also use and expect to continue to use third-party contract manufacturers to produce or assist in the production of certain of our large molecule marketed products as well as a number of our clinical product candidates. Manufacturing of Sensipar®/Mimpara®, our small molecule product, is currently performed by third-party contract manufacturers, except for certain finish activities performed by us in Puerto Rico.

The global supply of our products depends on actively managing the inventory produced at our facilities and by third-party contract manufacturers and the uninterrupted and efficient operation of these facilities. During the manufacturing scale-up process, and even after achieving sustainable commercial manufacturing, we may encounter difficulties or disruptions due to defects in raw materials or equipment, contamination or other factors that could impact product availability. (See Item 1A. Risk Factors — Manufacturing difficulties, disruptions or delays could limit supply of our products and limit our product sales and — We rely on third-party suppliers for certain of our raw materials, medical devices and components.)

Commercial Bulk Manufacturing

We operate commercial bulk manufacturing facilities in Puerto Rico and in several locations throughout the United States. (See Item 2. Properties.) We perform commercial bulk manufacturing for our proteins except Vectibix®, which is performed by a third-party contract manufacturer. We also supplement commercial bulk manufacturing for ENBREL, Prolia® and XGEVA® with a third-party contract manufacturer.

Commercial Formulation, Fill and Finish Manufacturing

We perform most of our commercial protein formulation, fill and finish manufacturing in our Puerto Rico facility. Formulation, fill and finish manufacturing for Nplate® and Vectibix® is performed by third-party

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Transcript of Stuart Watt

Date: September 23, 2019
Case: Genentech -v- Amgen (18-924-CFC)

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IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF DELAWARE

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GENENTECH, INC., and CITY OF HOPE

Plaintiffs,

-vs-

AMGEN, INC.

Defendant.

Case No.: 1:18-CV-00924-CFC

C.A. No. (17-1407-CFC) (Consolidated)

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Video-Recorded Deposition of STUART WATT

VOLUME I

Westlake Village, California

Monday, September 23, 2019

9:04 a.m.

Job No.: 263891

Pages: 1 - 302

Reported by: Tricia Rosate, RDR, RMR, CRR

CSR No. 10891

Video-Recorded Deposition OF STUART WATT,
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Pursuant to Notice, before Tricia Rosate, RDR, RMR, CRR, CCRR, Certified Shorthand Reporter No. 10891, in and for the State of California.