

THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

The opinion in support of the decision being entered today (1) was not written for publication in a law journal and (2) is not binding precedent of the Board.

Paper No. 33

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES

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Ex parte GEORGE N. PAVLAKIS and BARBARA K. FELBER

Appeal No. 1995-2723  
Application No. 07/858,747<sup>1</sup>

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ON BRIEF

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Before WILLIAM F. SMITH, ELLIS, and ROBINSON, Administrative Patent Judges.  
ROBINSON, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal from the examiner's final rejection of claims 1-11. Claims 12-33 stand withdrawn by the examiner as being directed to a non-elected invention and are not presented on appeal.

Claims 1, 6, and 10 are illustrative of the subject matter on appeal and are reproduced below:

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<sup>1</sup> Application for patent, filed March 27, 1992.

1. A method for reducing the effect of inhibitory/instability sequences within the coding region of a mRNA, wherein said effect of said inhibitory/instability sequences is a post-transcriptional effect, said method comprising the steps of:

- (a) providing a gene which encodes said mRNA;
- (b) identifying the inhibitory/instability sequences within said gene which encode said inhibitory/instability sequences within the coding region of said mRNA;
- (c) mutating said inhibitory/instability sequences within said gene by making multiple point mutations;
- (d) transfecting said mutated gene into a cell;
- (e) culturing said cell in a manner to cause expression of said mutated gene;
- (f) detecting the level of expression of said gene to determine whether the effect of said inhibitory/instability sequences within the coding region of the mRNA has been reduced.

6. The method of claim 1 or 2 wherein said mutating step changes the codons such that the amino acid sequence encoded by the mRNA is unchanged.

10. The method of claim 1 or 2 wherein said mRNA encodes the GAG protein of a Rev-dependent complex retrovirus.

The references relied upon by the examiner are:

- Hatfield et al. (Hatfield)                      5,082,767                      Jan. 21, 1992
- Schwartz et al. (Schwartz), "Distinct RNA Sequences in the *gag* Region of Human Immunodeficiency Virus Type 1 Decrease RNA Stability and Inhibit Expression in the Absence of Rev Protein," Journal of Virology, Vol. 66(1), pages 150-159, 1992.
- Wisdom et al. (Wisdom), "The Protein-Coding Region of *c-myc* mRNA Contains a Sequence that Specifies Rapid mRNA Turnover and Induction by Protein Synthesis Inhibitor," Genes & Development, Vol. 5, pages 232-243, 1991.

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Kunkel, "Rapid and Efficient Site-Specific Mutagenesis Without Phenotypic Selection," Proc. Natl. Acad. Sci. USA, Vol. 82, pages 488-492, 1985.

**GROUND OF REJECTION**<sup>2</sup>

Claims 1-11 stand rejected under 35 U.S.C. § 103. As evidence of obviousness, the examiner relies upon Schwartz, Wisdom, Kunkel and Hatfield.

We affirm the rejection.

**BACKGROUND**

At page 1 of the specification, applicants describe the invention as relating to methods of increasing the stability and/or utilization of mRNA produced by a gene by mutating regulatory or inhibitory/instability sequences (INS) in the coding region of the gene which prevent or reduce expression of the mRNA. The invention is also said to relate to constructs, including expression vectors, containing genes mutated in accordance with these methods and host cells containing these constructs. The methods are said to be particularly useful for increasing the stability and/or utilization of a mRNA without changing its protein coding capacity and are said to be useful for allowing or increasing the

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<sup>2</sup>The examiner and appellants both present arguments relating to the objection to the specification under 35 U.S.C. § 112, first paragraph as lacking support or antecedent basis in the application, as filed, for matter added by amendment to the specification. No claim is rejected on this basis. The Board of Patent Appeals and Interferences reviews adverse decisions of examiners in applications for patents (35 U.S.C. § 7(b)) on appeal from applicants whose claims have been twice rejected (35 U.S.C. § 134). Since no claim is rejected on this ground, review of this issue is not appropriate. Where the new matter is confined to amendments to the specification, review of the examiner's requirement for cancellation is by way of petition. See MPEP § 608.04(c).

expression of genes which would otherwise not be expressed or which would be poorly expressed because of the presence of INS regions in the mRNA transcript.

### **DISCUSSION**

#### Grouping of the Claims:

At page 6 of the Appellants' principal brief (Principal Brief), appellants state that the claims do not stand or fall together and state that claims 6-9 and 10-11 are separately patentable. Appellants have not separately argued the claims within each group. Therefore, we have separately considered the final rejection only as it applies to claim 1, as representative of claims 1-5, claim 6, as representative of claims 6-9 and claim 10, as representative of claims 10-11.

#### Claim Interpretation:

Claim 1 is directed to a method of reducing the effect of inhibitory or instability sequences (INS) within the coding region of a mRNA wherein the inhibitory or instability sequence results in a post-transcriptional effect. At page 2 of the specification, applicants state that the "post-transcriptional effect" includes:

nuclear post-transcriptional processes (e.g. splicing, polyadenylation, and transport) as well as cytoplasmic RNA degradation. All these processes contribute to the final steady-state level of a particular transcript.

We read this claim to encompass any effect which is observed to occur following transcription of the gene to generate the mRNA. The stability of the transcribed mRNA or

the ability of the mRNA to be expressed would be such effects. The method comprises the steps of providing a gene which encodes an mRNA, identifying the INS within the gene which encodes the INS within the coding region of the mRNA, mutating the INS within the gene by making multiple point mutations, transfecting the mutated gene into a cell, culturing the cell in a manner to cause expression of the gene and detecting the level of expression of the gene to determine whether the effect of the INS within the coding region of the mRNA has been reduced. Claim 6 depends from claim 1 or 2 and further limits the method by requiring that the change to the codons of the gene are such that the amino acid sequence encoded by the mRNA is unchanged. Claim 10, similarly depends from claims 1 or 2 and provides that the mRNA encodes the GAG protein of a Rev-dependent complex retrovirus.

The rejection under 35 U.S.C. § 103

Claims 1-11 stand rejected under 35 U.S.C. § 103 as being obvious over Schwartz in view of Wisdom, further in view of Kunkel and Hatfield.

The examiner has relied upon Schwartz as teaching (Answer, page 5):

[m]ethods of identifying RNA sequences in the Gag region of HIV-1 which decreases RNA stability and inhibit expression via deletion analysis wherein the inhibitory/instability sequences is (sic, are) detected by fusing a reporter gene (CAT construct), transfecting the reporter constructs into cells (see page 151, column 2, second paragraph) then detecting the level of expression of the reporter gene containing the inhibitory/instability sequences (see figure 3A-C). Schwartz et al. teaches that altering these inhibitory/instability sequences by deletion result in stable mRNA.

The examiner acknowledges that Schwartz does not teach (Answer, page 5):

[m]utating said instability/inhibitory sequences within said gene by making multiple point mutations such that the amino acid sequence encoded by mRNA is unchanged.

The examiner relies on Wisdom as teaching (Answer, page 6):

[i]nstability/inhibitory sequences coding for 4 amino acids of c-myc mRNA and disruption of the initiation codon by a single point mutation effects mRNA stability and rapid turnover of the mRNA . . . .

The examiner relies on Kunkel as teaching (Answer, page 6):

[a] method of producing several single base substitution mutations introduced within a DNA template region as a way of generating a high frequency of desired nucleotide changes of a genotype by using a uracil-containing DNA template, prepared by standard procedures after growth on an *Escherichia coli dut ung* strain (see page 488, column 1). Kunkel also teaches site specific misincorporation mutagenesis which is used to change single codons from C to A and C to T (see page 490, column 2).

Finally, Hatfield is said to disclose (Answer, paragraph bridging pages 6-7):

[a]nalyzing the frequency of at least some codon pairs (encoding the same amino acid) from organism to organism, this information is used to construct and express altered or synthetic genes having greater translational efficiency for highly expressed genes (see abstract). Hatfield et al. also discloses that by replacing from 10% to 20% or more of the codons may be designed to either increase or decrease the translational efficiency of the codon pairs (see column 5, lines 34-40).

The examiner concludes (Answer page 7):

[I]t would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to identify RNA sequences in the gag region of HIV-1 which decrease RNA stability or inhibit expression as taught by Schwartz et al, in view of other known instability/inhibitory sequences and

disruption of the initiation codon by a single point mutation altering mRNA stability which results in rapid turnover of the mRNA as taught by Wisdom et al, further in view of . . . methods for producing several single base substitution mutations introduced within a DNA template region as a way of generating a high frequency of desired nucleotide changes of a genotype as taught by Kunkel and analyzing the frequency of at least some codon pairs (encoding the same amino acid) as disclosed by Hatfield et al. to create a method for reducing the effect of inhibitory/instability sequences within the coding region of a mRNA as a whole for the expected advantages of increasing the stability and/or utilization of a mRNA produced which would otherwise not be expressed or which would be poorly expressed to achieve higher levels of expression.

The examiner bears the initial burden of presenting a prima facie case of obviousness. In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). On the record before us, we conclude that the examiner has made out a prima facie case of unpatentability of the claimed subject matter. **Both Schwartz and Wisdom are concerned with the identification** of those regions of a gene which express an unstable mRNA which contribute to that instability. Both make use of mutational means which would be expected to effect the expression of the INS region and both study the effects of such mutations to determine the effect on the mRNA stability and expression. It appears to us that Schwartz teaches everything except the multiple point mutation of the INS sequence. The use of a particular mutation process would reasonably appear to be an arbitrary matter of designer choice. The examiner has cited references which suggest that this mutagenesis method is known to those skilled in this art. In addition we note page 32, lines 10-25 of the specification where the appellants indicated that the particular mutagenesis process used in the claimed invention is known in the art.

We find this combination of evidence sufficient to establish a case of prima facie unpatentability of the claimed method.

Where, as here, a prima facie case of obviousness has been established, the burden of going forward shifts to the appellants. In re Piasecki, 745 F.2d 1468, 1472, 223 USPQ 785, 788 (Fed. Cir. 1984), In re Rinehart, 531 F.2d 1048, 1052, 189 USPQ 143, 147, (CCPA 1976).

In rebuttal, the appellants initially argue (Principal Brief, page 7) that:

Schwartz et al. do not teach or suggest using multiple point mutations to increase stability. . . Schwartz et al. do not suggest, contemplate, or teach the use of anything other than the Rev regulatory protein to reduce the effect of the INS present in HIV-1 gag gene. There is no hint that would lead one skilled in the art to know that multiple point mutations would reduce the effects of INS.

While we agree that Schwartz does not teach the use of multiple point mutations in the identification and study of INS regions in the HIV-1 gag gene, we do not read this disclosure to indicate that the Rev regulatory protein is the only factor important in reducing the effects of the INS region present in the gene. Schwartz described the effect of a series of the deletion mutagenesis which evaluated the ability of the modified gene to express stable mRNA both in the presence and absence of Rev. (Note, for example, page 154, column 2, first full paragraph). With regard to the use of multiple point mutations, we agree with the examiner that the combination of Wisdom and Kunkel, as discussed above, would have reasonably suggested the substitution of multiple point mutations for the deletion mutations used by Schwartz to determine whether a INS region was relevant to the stability of the mRNA being studied.

As to Wisdom, appellants urge that (Principal Brief, page 9):

Wisdom et al. do not teach or suggest using multiple mutations of the instability region to increase stability. Wisdom et al. is limited to the use of either (1) a single mutation in the translation initiation codon from ATG to ATC, which prevents translation of the mRNA or (2) the use of translation inhibitors to reduce the effect of the INS present in the c-myc gene. This is no hint that would lead one skilled in the art to know that multiple point mutations would reduce the effect of INS. ... Furthermore, there would be no motivation to use multiple point mutations to reduce the effect of the INS once the use of single point mutation (which prevents translation) or the use of translation inhibitors were shown to be effective.

We do not agree that Wisdom should be given such a limited reading. Wisdom does disclose that a single point mutation in the coding region of the gene effects translation and the stability of the resulting rRNA.

Appellants urge that:

[t]here are no teachings, explicit or implicit, in either Schwartz et al. or Wisdom et al. which would lead one of ordinary skill in the art to combine their teachings with Hatfield et al., to generate Applicants' invention.

We are not persuaded. As we have stated, both Schwartz and Wisdom had suggested teach the use of mutagenesis of the INS region of a gene to study the effects of the mRNA which result from the transcription of that gene. This the modification of nucleotide sequence in the INS region of the gene is intended to modify or eliminate the instability or inhibitory effect of that part of the resulting mRNA. Thus, whether one of ordinary skill in the art chose to modify, through mutagenesis, the INS region of a gene using deletion mutation or multiple point mutation in order to reduce the inhibitory or instability causing effect of this

region of the gene is merely a matter of choice where the choice is made from among those techniques recognized by the prior art.

We similarly disagree with appellants' contention that Schwartz and Wisdom teach away from the present invention in suggesting that protein interactions may be or are responsible for the effects of the INS. While each of the references discuss the involvement of other proteins in the process, a reasonable reading leads one to the conclusion that it is the INS region of the gene which ultimately dictates whether these proteins effect the stability of the mRNA or its ability to express the relevant protein.

Appellants have additionally addressed the subject matter of claim 6 arguing that Wisdom and Schwartz do not teach a process where the multiple point mutations do not change the amino acid sequence encoded by the mRNA. While Schwartz does not explicitly describe a mutation which does not change the amino acid sequence encoded by the mRNA, claim 6 reads on changes in a silent codon region as described by Hartfield or a change in the 3' untranslated region as suggested by Schwartz. (Note page 152, column 2, last paragraph). The result of either, would be a mutant which would not effect the amino acid sequence encoded by the mRNA. Similarly, in urging the separate patentability of claim 10, appellants content that Schwartz, at page 755, Col. 1, lines 18-23, teaches the expression of gag protein which is Rev dependent while the specification teaches expression in a Rev independent manner. However, the portion of the reference cited relates to the expression of the p17<sup>gag</sup> protein where the gene has not been modified. Schwartz indicates that the test was performed only to verify the hypothesis that the INS

region was present in the coding region of p17<sup>gag</sup>. We note page 154, column 2, lines 21-23 which indicates that the expression of the plasmid pTRG(481-631) "expressed high levels of mRNA both in the absence and in the presence of Rev. . ." Thus the reference describes at least one mutated p17<sup>gag</sup> gene which results in mRNA which was Rev independent as compared to similar plasmids which were Rev dependent. Thus, Schwartz describes the process as it relates to the GAG protein of a Rev-dependent complex retrovirus as claimed.

Appellants also urge that the invention must be considered as a whole and that even if the individual steps were taught, the multi-step method and the elements used are unobvious. However, this is not the situation here. The prior art relied upon by the examiner is not merely a collection of the individual steps or elements. Schwartz teaches all aspects of the invention as claimed except for the use of a mutagenesis process involving multiple point mutations. Yet as we concluded above, the substitution of this mutagenesis technique for the deletion technique used by Schwartz would have been obvious to those skilled in this art based on the disclosure of the references cited by the examiner. For the same reasons, we do not agree that the examiner has improperly made use of hindsight construction of the invention.

Having carefully weighed the evidence in favor of patentability against the evidence against patentability, we conclude that the examiner has established a prima facie case of unpatentability of the claimed subject method which appellants have not overcome by

arguments or evidence. Therefore the rejection of claims 1-11 under 35 U.S.C. § 103 is affirmed.

**OTHER ISSUES:**

In addition, should further prosecution occur in this application, we urge the examiner to consider whether the principles set forth in the decision of University of California v. Eli Lilly & Co., 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir.), reh'g denied (en banc), 1997 U.S. App. LEXIS 31640 (1997), cert. denied, 118 S. Ct. 1548 (1998), and Enzo Biochem Inc. v. Calgene Inc., \_\_\_ F.3d\_\_\_, 52 USPQ2d 1129 (Fed. Cir. 1999), rendered by the Court of Appeals for the Federal Circuit since the filing of this appeal are applicable to the current claims. In these decision, the court makes clear that the consideration of claims directed to genetic materials differs from consideration of claims directed to chemical materials in determining whether the claims are in compliance with the written description requirement of 35 U.S.C. § 112, first paragraph and carefully delineates how the analysis is to be made. The disclosure of the instant specification is limited to mutation of a limited number of gag genes from HIV-1 virus. It is not readily apparent to us that results observed on this limited scale could reasonable be practiced by those skilled in this art to the scope of subject matter claimed without undue experimentation. As explained in PPG Indus., Inc. v. Guardian Indus. Corp., 75 F.3d 1558, 1564, 37 USPQ2d 1618, 1623 (Fed. Cir. 1996):

In unpredictable art areas, this court has refused to find broad generic claims enabled by specifications that demonstrate the enablement of only one or

a few embodiments and do not demonstrate with reasonable specificity how to make and use the other potential embodiments across the full scope of the claims. See, e.g. In re Goodman, 11 F.3d 1046, 1050-52, 29 USPQ2d 2010, 2013-2015 (Fed. Cir. 1993); Amgen, Inc. v. Chugai Pharmaceutical Co., 927 F.2d 1200, 1212-14, 18 USPQ2d 1016, 1026-28 (Fed. Cir.), cert. denied, 502 U.S. 856 (1991); In re Vaeck, 947 F.2d 488, 496, 20 USPQ2d 1438, 1445. Enablement is lacking in those cases, the court has explained, because the undescribed embodiments cannot be made based on the disclosure in the specification, without undue experimentation. But the question of undue experimentation is a matter of degree. The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation "must not be unduly extensive." Atlas Powder Co., v. E.I. DuPont De Nemours & Co., 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984). The Patent and Trademark Office Board of Appeals summarized the point well when it stated:

The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. Ex parte Jackson, 217 USPQ 804, 807 (Bd. App. 1982).

In addition, the examiner should consider whether "the level of expression of said gene" would reasonably serve as a means of evaluating whether the effect of the INS within the coding region of the mRNA has been reduced. Both Schwartz and Wisdom would appear to suggest that the level of transcription of the mRNA would not necessarily reflect the "post transcription effect" called for by the claims.

**SUMMARY:**

The rejection of claims 1-11 under 35 U.S.C. § 103 is affirm.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

**AFFIRMED**

WILLIAM F. SMITH	)	
Administrative Patent Judge	)	
	)	
	)	
	)	BOARD OF PATENT
JOAN ELLIS	)	
Administrative Patent Judge	)	APPEALS AND
	)	
	)	INTERFERENCES
	)	
DOUGLAS W. ROBINSON	)	
Administrative Patent Judge	)	

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