

failed to address an important issue in this case – the motivation for misconduct when results from repeated experiments were available and the fact that none of the allegations affected the central theme and results of the paper. What was more farcical was their repeating of the original allegations and their claim to have unearthed more misconduct than what *JBC* had found.

This whole episode has caused me inexpressible anguish, apart from irreparable damage to my career. There is no doubt in my mind that I have been wronged under the guise of scientific integrity. What has kept me going is the unshakable trust several people have in me and their shared sense of outrage – I am very thankful for it.

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The NCCS case

In recent times there have been a spate of write-ups expressing concern on the ethics of scientific research among the Indian scientific community. This relates to plagiarism, data manipulation and falsification. One such case relates to that of Gopal Kundu and his colleagues at the National Centre for Cell Science, Pune. I happened to chair a committee that investigated the allegations against Kundu and colleagues that they have used the same data in two different publications in the *Journal of Biological Chemistry (JBC)*, describing different treatment conditions. The committee came to the conclusion that manipulation of data was not evident based on the information provided and exonerated the team involved. In the light of all the subsequent newspaper reports and emails, I thought I should clarify to the scientific community, the basis on which my committee exonerated the team of wrongdoing.

The episode began with emails in the names of Shivaji Bhode and Ganapati Mahabaleswar being received by G. C. Mishra, Director, NCCS (a DBT institute) that the two papers published by Hema Rangaswami *et al.* in the *JBC* (refs 1, 2 above) contained the same data; the

data from the earlier paper was manipulated to describe the results on the responses of different pathways in the subsequent paper.

Mishra informed me that an internal committee had felt that a *prima facie* case exists and, therefore, an external committee should investigate the allegations in detail. My committee consisted of six reputed, active researchers from different institutions in the country as its members. The committee met at NCCS, Pune on 13 August 2006 to examine the allegations, the details of which were made available to the committee earlier. There were nine points in the allegations, each involving mostly Western but also other types of blots. The bands in the blots were subjected to computer analysis. These included the zoom blots of each band, colour cube analysis and lab image 1D-2006 professional software analysis. Original ECL/autoradiograms for six of the nine figures under consideration and the powerpoint pictures of the originals for the remaining three points were provided to the committee by Hema Rangaswami who came from the US to appear before the committee. The committee also had access to the note books where data on experimental repeats of the figures presented in the papers were available. All members of the laboratory, including those not part of the papers, were examined individually.

The committee did not find evidence of manipulation and came to the conclusion that the emails had been sent with a malicious intent of bringing disrepute to NCCS. A brief report was submitted to the Director, exonerating the investigators of any manipulation of data between the two papers.

In the meanwhile, *JBC* had also apparently received emails on the manipulations and on the basis of its own investigation decided to withdraw the *JBC* (2005) paper. In a subsequent input to *Science* (ref. 7, above), Shelag Ferguson-Miller, chair of *JBC*'s publication committee stated that a computer analysis found that two control blots were identical to images that had been labelled differently in the 2004 publication and it seemed that there had been deliberate misrepresentation. This involved two figures that pertained to two of the nine allegations. Kundu had sent replies to both these allegations, enclosing all the data. However, *JBC* chose to remain silent and sent back the data without any comments.

Based on a written complaint received from Sohan Modak, the Society for Scientific Values (SSV) claims to have carried out its own analysis and found Kundu guilty. It also indicates of possible complicity of my committee in the exoneration of Kundu. It basically repeats some of the original allegations and the analysis is available on its website.

Our original report was brief but based on extensive analysis. But, in the light of the unexpected turn of events, the Department of Biotechnology, Government of India has sought a detailed report from the committee. Without getting into all the details, I would like to highlight some of the crucial facts.

I am not a computer expert and although computer analysis of the bands showed that they are not reproductions or manipulations of a single figure, I was not convinced. In my opinion, the output of the computer depends on what is fed in. Thus, analysis of the published figures as such would not add to much, since the original allegations are based on such an analysis. What tilted the opinion of the committee in favour of Kundu, was the availability of the original ECL autoradiograms from which the strips (rows, left to right) were cut. I just want to give one example to highlight the issue. This relates to one of the points of the original allegations and one of the two points on the basis of which *JBC* decided to withdraw one of the papers. In the Figure, the top three strips are images of the original autoradiograms. Although the pattern of intensity changes between the bands (L to R) is similar in all the three cases, it is obvious that the data are from three independent autoradiograms. In fact, one can see an artifactual faint band appearing below the bands of interest in the top left strip and a smudge appearing in some of the lanes above the bands of interest in the bottom third strip. When these strips are processed to highlight only the bands of interest using Photoshop, all the three look identical as given in the bottom part of the figure (below the line). In fact, whole autoradiograms would reveal other artifacts away from the region of interest and it looks it is better not to have clean autoradiograms to prove to the world that data are not duplicates of the same! Top journals demand huge amount of data and there is need for data compaction which results in lanes and rows of gel data from different experiments being lined-up in a sin-

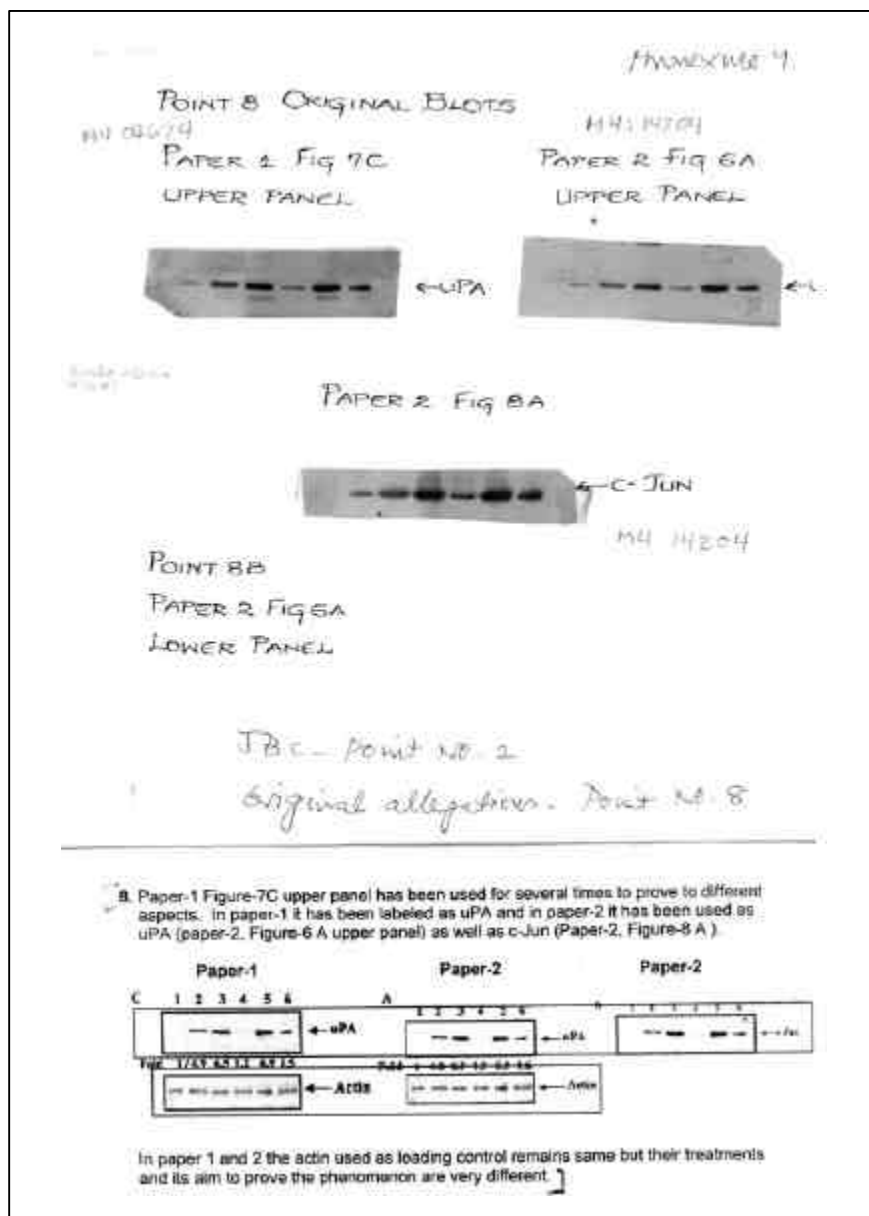


Figure.

gle figure. There is no space in journals to provide whole autoradiograms. The fact of the availability of original autoradiograms from which the strips are cut and processed, is itself a proof that a single figure has not been duplicated or manipulated.

A second important fact is that five of the nine original allegations relate to loading controls and one of the two figures leading *JBC* to withdraw the paper is also a loading control! To the non-biologists, I would like to explain that loading controls ensure that the same amount of protein (say JNK) is loaded in all the wells. The substantive part is actually the phosphorylation status of the protein that could vary between treat-

ments. Thus, while loading controls would show more or less the same intensity of bands, the bands depicting the phosphorylated protein would show dramatic differences in intensity depending on treatment. It is beyond anybody's imagination, why would anyone need to duplicate loading controls. Plenty of such strips would be available and if anyone wants to cheat, there is absolutely no need to duplicate or manipulate bands depicting loading controls, since they are all expected to be of similar intensities! *Even here, the analysis did not reveal any manipulation.*

Why do the bands in different lanes seem to have similar shapes including a dot or two towards the edges? This arises

from the use of the same gel apparatus and comb. The slots in the comb are not really identical but are with some invisible notches here and there. This leads to typical artifacts and if the same investigator runs these gels right through, band shapes along with the artifacts look very similar.

The other original allegations pertain to flipping the same blot and making a 5 slot blot into a 4 slot blot. However, the allegations are not substantiated when one looks at the original autoradiograms.

Three professors from the University of California, San Diego (the institution at which the first author Hema Rangaswami is currently located) have also looked at the data. The gist of their conclusions is as follows: Even with a lot of imagination it is difficult to envisage re-arranging of lanes, since the shapes and intensities of the supposedly related lanes differ significantly and there is no break in the background to indicate that cutting of lanes has taken place. The two *JBC* papers describe two parallel, very similar, but yet independent pathways that regulate *c-jun* downstream of osteopontin binding to its integrin receptor. Many of the experiments presented are similar in organization and it is not surprising that the pattern of Western blots describing downstream effects of constitutively active or dominant negative molecules is similar. These professors note that the accusations are untenable and outrageous.

I once again want to reassure the scientific community that my committee did not take the investigation lightly, but put in all efforts to evaluate the truth on the basis of data available. It was also evident that even if some minor flaw had escaped the committee's attention (one was literally looking at the similarities of over 100 bands *and we could not establish manipulation*), the conclusions of the two papers would not in any way be affected. I must confess that I was also deeply concerned with the career of bright young scientists and a host of students, but not at the expense of condoning a fraud. I want to plead with anyone professing on the primary concern of ethics to also consider the career and pride of competent young scientists and not to hoist them on the lamp-post with preconceived ideas or to settle scores with the establishment. Scientists are generally left to fend for themselves, and the colleagues in the country in general keep

mum, even when they are aware that their colleague is unfairly treated, for fear of being dragged into the quagmire. To attack the committee with credible scientists is also unfair and it would only drive the community to its shell, leaving public causes to vested interests. The committee stands by its earlier decision of exonerating Kundu and colleagues of any manipulation between the two papers.

I would like to make an appeal to the *JBC* to rescind its decision to withdraw the *JBC* (2005) paper.

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Response (Modak)

G. Padmanaban (GP) makes a great fuss about his committee's analyses of pictures of Western blots. However, his descriptions show that he has cleverly obfuscated the issue. For example, does a gel band have features? It is shocking to read his explanation, which is not scientifically acceptable. Here, I reproduce a composite of Western blots (Figure 1, based on refs 1 and 2 above). A densitometric comparison of these two, carried out using MATLAB software is available (in ref. 3 above). Rangaswami *et al.* claimed that the two Western blots represent two different experiments on two different proteins. Actually, a mere look at the gel pictures should be enough to any experienced and intelligent scientist to recognize the identical features.

It is true that a bland band cannot be easily questioned. However, if it has intrinsic features such as a double hump and so on, it is not humanly possible to have two identical bands. The way to distinguish them is to do computer analysis of the digitized images, which is what SSV did (ref. 3 above). I cannot accept that small noise bands are due to defects/grooves in the gel, gel support or comb. These are invariably stray artifacts, which, by definition, are random and their spontaneously repetitive occurrence in gels has a possibility less than a snowflake in the desert. Thus, there is more than sufficient evidence that the committee of six chaired by GP, the seventh, has

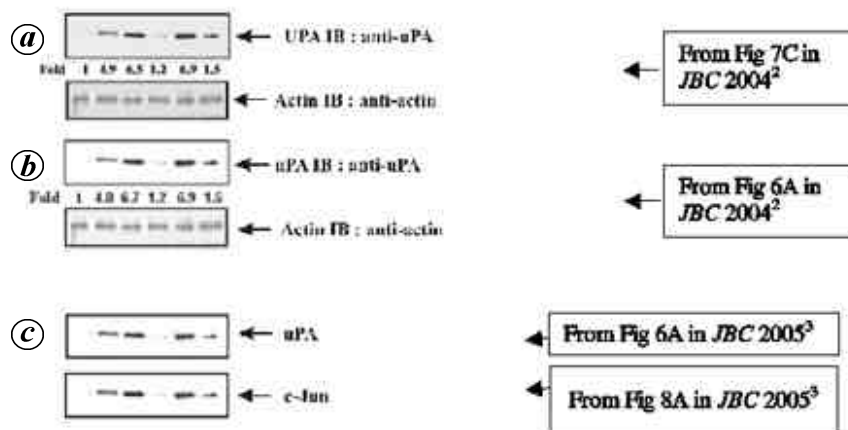


Figure 1. The top (a) two Western blot strips represent detection of differences in expression of UPA 1B as compared to the control for Actin 1B as shown in Figure 7c of Rangaswami *et al.* (ref. 1 above). The same strips are shown (b) to represent the same polypeptide in Figure 6A of Rangaswami *et al.* (ref. 2 above). Note that the authors insinuate small differences in the amount of UPA 1B expressed in these gels, and the near identity is suggestive of the same experimental blot being shown in two different papers. In the bottom (c) two western identical blots are shown to represent entirely different proteins. Note the total identity in the shape, size and intensity of each band pair.

mis-stated the obvious. GP attempts escape by stating that he is no computer expert, but neither does he tell us who did the professional computer analysis nor does he provide the results of such analysis for open scrutiny. It is also unfortunate that the Padmanaban committee pretends not to understand 3-dimensional morphology and morphometry of bands in a gel that require quantitative analyses of shapes, sizes, volumes and patterns.

GP is correct up to the point that the output of the computer depends on what is fed in. Thus, according to him, *JBC*-SSV allegations, based on analysis of the published figures as such, would not mean much and that the lab records and raw data were more important. He saw nothing wrong in the fact that the raw data was not available in Kundu's lab at the time of the first enquiry and had to be presumably brought from the USA by Hema Rangaswami! Nonetheless, the records hurriedly produced were sufficient to convince the GP committee, who did not even care to undertake forensic analyses by dating these.

GP's subterfuge over controls and experimental blots is equally intriguing, especially considering that three out of seven re-used figures clearly listed and established by SSV (ref. 3 above) deal with experimental blots. In contrast, GP's report or letter does not clearly define the specific figures investigated by his committee. I am wonderstruck by GP's belief

that there is no need to reproduce data and controls in every experiment when so many gels would be easy to lay hands on and their misuse will not change the conclusions. Brutally stated, when a control is found to be false, the clock strikes thirteen, as noted by Mark Twain, meaning all that happened before and after is equally unreliable.

It is important to mention that, while GP's committee fully exonerated Kundu, *JBC* has withdrawn the 2005 paper (ref. 2 above), published an editorial against image manipulation and also banned manipulation of digital image data (ref. 5 above). It is also pertinent that the GP committee owes its very origin to Kundu's withdrawal of a written confession to the first enquiry committee, practically admitting that misconduct occurred and that the published papers would be retracted. GP never worried as to why such a confession was given and what was the nature of the 'duress' cited by Kundu and the motives for its withdrawal. I wonder whether, like Maradona's (in)famous goal in the Football World Cup final, there also was 'The Hand of God' controlling the psyche of the committee of *The Magnificent Seven*.

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