# Loss of kinase activity

SIR — Vetrie *et al.*[1] showed that a novel intracellular protein-tyrosine kinase is involved in X-linked agammaglobulinaemia (see also ref. 2). They related two point mutations which cause Lys 430→Glu and Arg 525→Gln exchanges to loss of kinase function and in turn to the expression of the immune deficiency disease.

Our own data provide a basis specifically to assess the function of the residues Lys 430 and Arg 525 in the newly discovered *atk*-tyrosine kinase. Recently, we solved the 2.0-Å crystal structure of the protein kinase A catalytic subunit from porcine heart in a complex with a nucleotide triphosphate and a pseudo-substrate[3], and suggested a mechanism for phosphotransferase and substrate binding of protein kinases. Residue Lys 72 of protein kinase A corresponds to Lys 430 in *atk* and is invariant in all protein kinases[4]. This residue is involved in MgATP binding[5], and a direct role in catalysis has often been proposed. We found, however, that Lys 72 is too far away from the catalytic site to participate in the immediate catalytic act, but the residue does bind MgATP, as it coordinates oxygens of the $\alpha$- and $\beta$-phosphoryl groups. A glutamate substitution is likely seriously to interfere with ATP coordination.

Carrera *et al.*[6] proposed a seemingly contradictory role of the conserved lysine in catalysis and not in ATP binding, mainly because the affinity of 8-azido-[$\gamma$-$^{32}$P] ATP for the tyrosine kinase pp56$^{lck}$ was not significantly changed after mutating the lysine to arginine, whereas kinase activity was lost. However, the arginine must not necessarily affect the affinity for the triphosphate, but could critically disturb its stereochemistry, leading to dislocation of the $\gamma$-phosphate.

Residue Lys 168 of protein kinase A, which has not yet been considered to have a general role in catalysis as it is conserved only in Ser/Thr-protein kinases, appears to have an important function in the immediate process of phosphotransfer through stabilizing the transition state. Its positive charge is located within hydrogen bonding distance of the $\gamma$-phosphate and in the vicinity of the substrate hydroxyl group. In tyrosine kinases two sequences are found at this relative position, either **Arg**(168)-Ala-Ala, or Ala(168)-Ala-**Arg** (ref. 4). Because an extended residue at position 170 (corresponding to Arg 525 in *atk*) would place its charge in a position occupied by the Lys 168 charge in protein kinase A if the position corresponding to 168 is small and hydrophobic, it is likely that this spatial position of a positive charge is conserved among all protein kinases. The correct alignment as given in ref. 4 should therefore not be adjusted to align the alanine pair into homologous positions.

We suggested that in tyrosine kinases the Lys 168-homologue is functionally replaced by an arginine. The important functions of these residues in ATP binding and in the catalytic process, respectively, explain the loss of protein-kinase activity in Lys 430 and Arg 525 mutants of the *atk* kinase.

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# No HMG-1 box signature

SIR — There have been several recent reports of the cloning and sequencing of a complementary DNA encoding a protein, CCG1, which is thought to be involved in the progression of cells through G1 phase of the cell cycle[1] and which has been shown to be one of the TATA-binding protein (TBP)-associated factors (TAFs) in transcription initiation complexes in humans[2,3]. Indeed, a functional homologue of this protein, $TAF_{II}250$, has been isolated from the *Drosophila* TFIID complex[4]. The current model for the function of this $M_r$ 250,000 protein is that it is a central core for several protein–protein interactions with other TAF proteins[4]. I was surprised to find that the CCG1 protein does not contain an HMG-1 box domain, as previously noted[1].

HMG-1 box domains have been found in many proteins involved in transcription regulation as well as in other cellular functions[5–7]. HMG-1 box domains usually contain a regularly spaced pattern of aromatic residues with no gaps needed for alignment. Furthermore, at a variable distance towards the amino-terminal end of the domain, there is a hydrophobic region in which at least two out of five amino acids are aromatic. This is best represented by the regular expression or signature shown in the box above[7].

A search of the protein sequence databases using this sequence as the query gives a list of proteins containing an HMG-1 box domain, which agrees with a similar list obtained using software such as the Blast network service at the NCBI (ref. 8).

I have examined the human CCG1 gene product sequence by several methods. An initial screening identifies several regions of low compositional complexity (regions of locally 'biased' amino-acid composition such as polyglutamine and basic regions), including the central region of the proposed HMG-1 box in CCG1 (residues 1,165–1,205)[9]. Furthermore, this same region is a statistically significant mixed-charge segment[10]. This would explain the initial erroneous statement that this sequence is similar to the charged HMG-1 box domain. A search of the NCBI non-redundant protein sequence database using Blast software[8] reveals that the only domain with any similarity to other proteins in the database is the region between residues 1,336 and 1,529, which contains two repeats of a previously identified bromodomain[3,11]. Regions in this domain show similarity to several proteins including GCN5, SPT7, STH1, NPS1, SNF2/GAM1 and YK107 from *Saccharomyces cerevisiae*; human peregrin and RING3; and *fsh* from *Drosophila*. No statistically significant similarities with any of the numerous (more than 130) HMG-1-box-containing proteins in the database are detected. There are also two repeats of the bromodomain in the *Drosophila* $TAF_{II}250$ protein[4].

These findings do not support the hypothesis that there is an HMG-1 box domain in these proteins or that this is the domain that has DNA-binding capabilities. In addition, CCG1 protein has also been reported to share sequence similarity with NF-κB, SWI4 and bacterial $\sigma$-factors[3]. None of these similarities is significant when analysed by quantitative methods.

**[GSA][YF]..[YFW].*[GSA]..[WYF].....[KRQ]..[YFW].....[KRQ]..[YFH].....[YFW]**

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