Section I - Detailed description of the image-tests

Section II - Qualitative analysis of the realistic image ("unfolding" exercise)

Section I – Detailed description of image-tests

Test-1

The below text and figures were used in an 80-minutes image test (figures S1-4).

Text

The adrenaline to glycogen phosphorylase image-test

When adrenaline, released by the adrenal glands, binds to receptors on skeletal muscle cells, it causes the rapid degradation of glycogen in many molecules of glucose-1-phosphate. The latter, after conversion to glucose-6-phosphate enter glycolysis and this leads, if the need is there, to a highly elevated production of ATP. This process ensues after an environmental stimulus (e.g. fear) and allows for an appropriate response of the organism (fight or flight). The binding of adrenaline to its receptor (βAR) leads to the activation of a GTP binding protein (a heterotrimeric G-protein). This protein is composed of three subunits, Gα, Gβ and Gγ. The occupied adrenergic receptor drives the exchange of GDP against GTP in the Gαs subunit, leading to its dissociation from the trimeric complex and subsequent binding to adenylyl cyclase, a membrane-bound enzyme. This causes the activation of adenylyl cyclase and leads to the conversion of ATP into a second messenger called cyclic-adenosine-monophosphate (cAMP) (with the loss of two phosphates (2Pi)). Numerous copies of the second messenger diffuse into the cell and bind a regulatory subunit (RIα) of the serine/threonine protein kinase A (PKA). As a consequence, RIα detaches and this frees the catalytic site of the protein kinase (allowing the kinase activity to express itself). Its substrate is yet another protein kinase, called phosphorylase kinase. This kinase is composed of a tetrameric protein complex, with subunits α, β, γ and δ. The regulatory subunits α and β are phosphorylated. How this leads to the activation of the catalytic subunit (the γ subunit) remains to be determined. Phosphorylase-kinase finally phosphorylates the glycogen degrading enzyme glycogen-phosphorylase. The addition of a single phosphate (covalently bound to serine-14) has important consequences for the conformation of the catalytic site of glycogen phosphorylase. It causes the displacement of a loop of nine amino acids, going from a "tense" to a "relaxed" state. In the "tense" state, these amino acids impede access to the catalytic site and therefore prevent degradation of glycogen into glucose-1-phosphate. Moreover, phosphorylation on serine-14 renders the enzyme less sensitive to allosteric inhibition, normally imposed by glucose-6-phosphate (an "end-product" inhibitor) or ATP (an indicator of the abundance of "fuel"). In this pathway, adrenaline interferes with cellular homeostasis and prepares (in advance) the muscles for a fight or flight response.

The role of the ATP in regulation of enzyme activity

In the process described above, the role of ATP resides in the provision of a phosphate, which, after being

transferred onto an amino-acid, changes protein conformation and thus leads to a change in activity (being it activation or inhibition). ATP is very useful because the third phosphate (in the γ-position) is easily released and therefore the process of transferring a phosphate is energetically favorable under the right circumstances (catalyzed by a protein kinase). ATP is the most commonly used triphosphate-nucleotide (amongst GTP, CTP, UTP, TTP) for donation of phosphate but some kinases use GTP or CTP. Understand that ATP does not supply the energy to "activate" proteins (and bring them in vibrating or humming states)!

The cascade phenomenon and modularity of signaling pathways

Complex signalling cascades as described above have several "raisons d'être": Firstly, they allow a significant amplification of the signal emitted by receptors. In the end, only a few molecules of adrenaline can activate a large number of glycogen phosphorylase, thus ensuring an adequate cellular response. Secondly, the cascade allows several levels of intervention by other signaling pathways (cross-talk). For example, Ca^{2+} (which enters the myocyte following an action potential initiated by acetylcholine) interferes at two levels, increasing the activity of adenylyl cyclase and of phosphorylase kinase. Finally, it should be kept in mind that signalling pathways are not "designed" for a specific purpose, this complex pathway simply happens to work (sensitive and robust) and evolution has preserved it. Very often signalling components are employed for the regulation of numerous cellular processes. Industrial production has adopted a similar modular strategy; different parts can be used for different purposes. Specificity of the cellular response (secretion, contraction, gene expression etc), depends on the receptors expressed, their subcellular compartmentalization, the exposure to first messengers (hormone, neurotransmitters, adhesion molecules and others) and importantly the effectors modified by the pathway.
End of text

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Images of test 1 adrenaline-mediated activation of glycogen-phosphorylase

Figure S1 (realistic rendering)

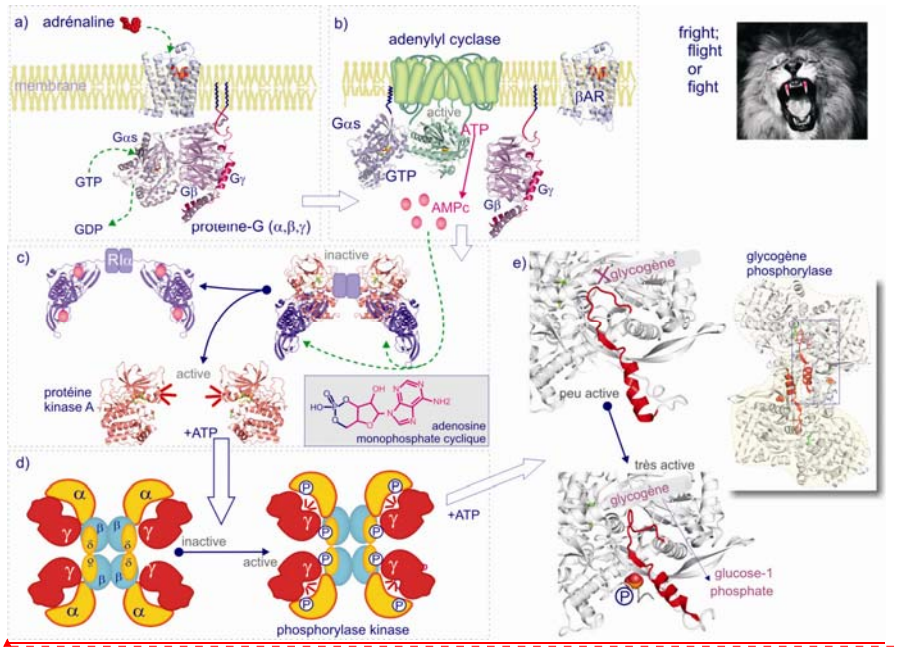

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Figure S2 (realistic-schematic rendering)

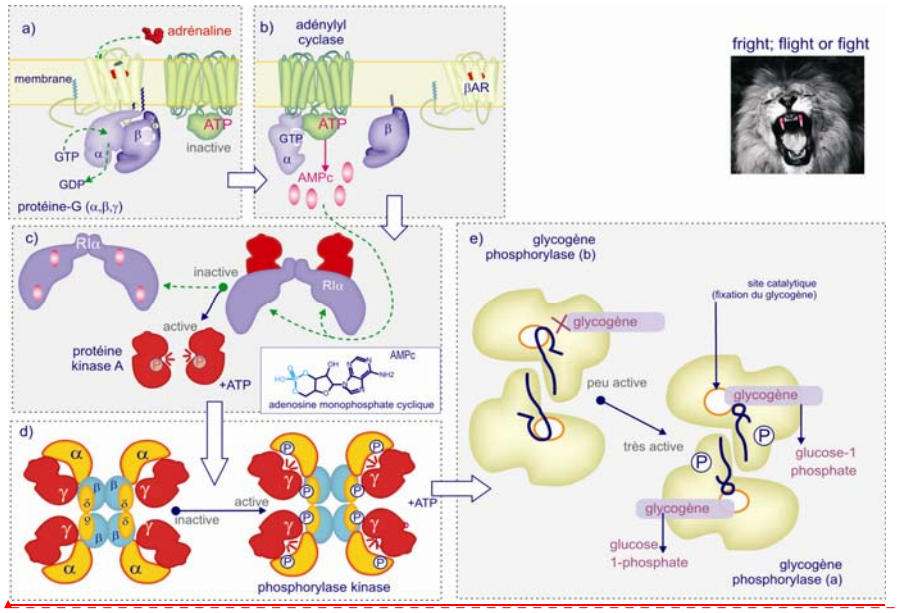

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Figure S3 (schematic rendering)

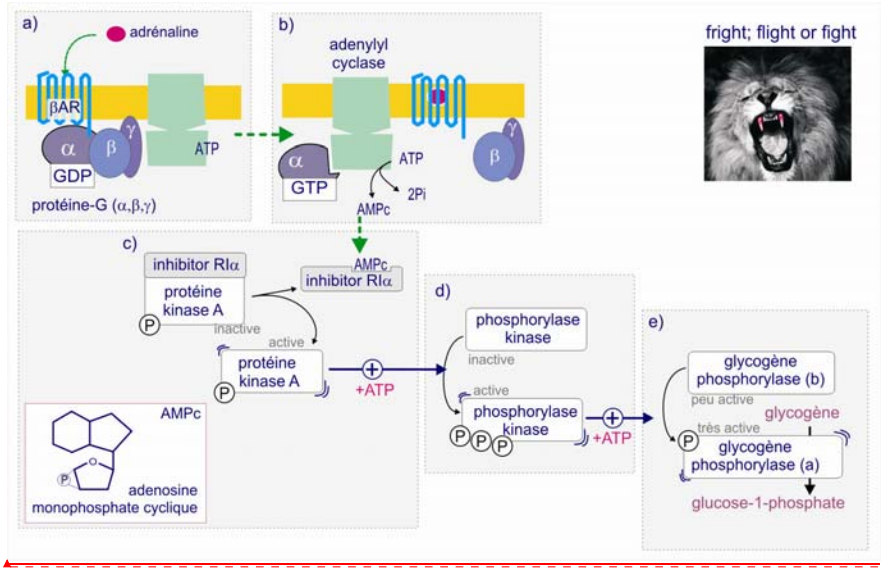

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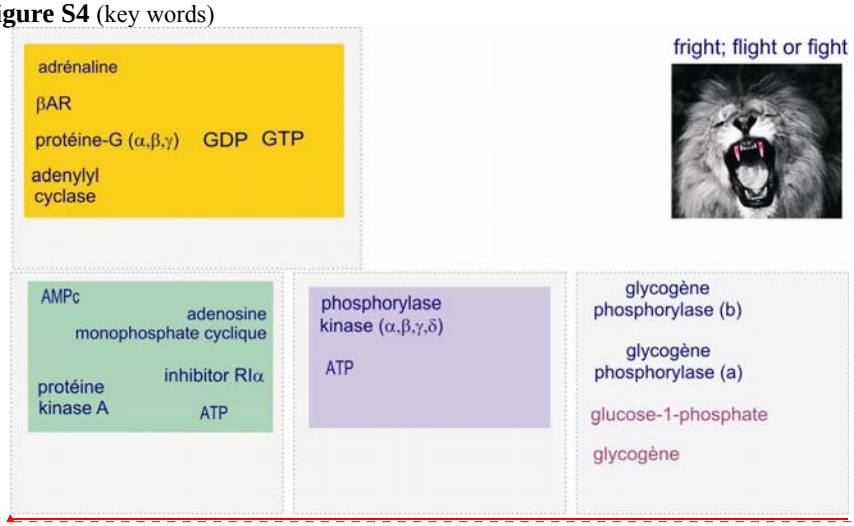
Figure S4 (key words)



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Slide projected during the reading section of test 1

<u>The next subjects will be covered by the test:</u>

- the whereabouts of glycogen (storage, metabolism)
- the sequence of events of the signalling cascade
- the nature of the components (protein, lipid, nucleotide, fatty acid)
- molecular detail of the activation processes
- you will be asked to draw the signalling cascade in your own style

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Question sheet of test 1

Name / first name group :

Fill in the box with the correct (or incorrect) proposition. Only one possibility per question.

1) glucose is stored as a polymer in the form of : *(1 point)*		a) fat in the muscle
	■	b) glycogen in the liver and in muscles
		c) glycogen in the blood
		d) fat in adipose tissue
		e) nucleotides in the nucleus
2) glycogen is degraded into : *(1 point)*		a) acetyl-CoA by glycogen-acetylase
	■	b) multiples glucose-1-phosphates by glycogen-phosphorylase
		c) multiples saccharides
		d) pyruvate
		e) short carbohydrate chains such glycosaminoglycans
3) adrenaline actives glycogen-phosphorylase in muscle via a signalling cascade that contains: *(1 point)*		a) a monomeric GTP-ase (like Ras)
		b) MAP-kinase
	■	c) adenylyl-cyclase
		d) the EGFR tyrosine kinase
		e) ubiquitin E3-ligase
4) GDP and GTP are : *(1 point)*		a) respectively, two or three times unsaturated fatty acids
		b) G-proteins
	■	c) nucleotides, respectively carrying two or three phosphates
		d) sugars rich in phosphates
		e) phosphorylated forms of glycogen
5) adenylyl-cyclase is : *(1 point)*		a) a GTP-binding protein
	■	b) an enzyme that converts ATP into cAMP + 2Pi
		c) an enzyme that inhibits protein kinase A (PKA)
		d) a cytosolic protein
		e) an enzyme that converts GTP (carried by Gα into cAMP + 2Pi
6) the second messenger cAMP : ***(attention, fill in the box with the incorrect answer**, 1 point)*	■	a) is a protein that binds and subsequently activates PKA
		b) liberates the PKA from its inhibitory regulatory subunit RIα
		c) allows, through the production of multiple copies, amplification of a receptor-mediated signal
		d) is a nucleotide
		e) allows the transmission of a membrane-located signal into the cell
7) the activation process of heterotrimeric G-proteins (Gα,β,γ) comprises: *(1 point)*		a) binding of adrenalin to its subunits
	■	b) an exchange of GDP against GTP on the Gα-subunit, leading to the dislocation of Gβγ and subsequent interaction of Gα with effector proteins
		c) recruiting the complex to the membrane through attachment of fatty acid chains
		d) the phosphorylation of GDP into GTP followed by conversion into cAMP
		e) the exchange of GDP against cAMP
8) glycogen-phosphorylase exists in two different states; little active and active. What is the underlying molecular mechanism? *(1 point)*	■	a) in its inactive form, a loop of 9 amino-acids impedes access of glycogen (substrate) to the catalytic site
		b) the active state is obtained by the energy input provided by the ATP
		c) the inactive state is obtained through hydrolysis of ATP
		d) the enzyme is constitutively active in its dephosphorylated state
		e) in the inactive form ATP impedes access of glycogen to the catalytic site

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9a) Draw the cascade of events, starting with the binding of adrenaline to its receptor and finally leading to the activation of glycogen phosphorylase. Remember, if a membrane is involved, do not forget to show it in the drawing! *(12 points)*

Five steps, 2 points each, if partially correct a single point per step
- binding of adrenaline to its receptor and exchange of GDP against GTP,
- dissociation of heterotrimeric complex and Gα.GTP-subunit binding to adenylyl cyclase
- production of cAMP leading to the removal of the inhibitor (RIα) and subsequent activation of PKA
- phosphorylation and activation of phoshorylase kinase
- phosphorylation and activation of glycogen phosphorylase

2 discretory points (carefulness of drawing, use of colours or others)

9b) starting from the occupied receptor, which processes in the cascade can amplify the signal? (make reference to your drawing)
(3 points)
- One receptor can interact and activate several G-proteins
- Adenylyl cyclase makes numerous copies of the second messenger cAMP
- PKA phosphorylates more than one phosphorylase kinase

(NB phosphorylase kinase and glycogen phosphorylase are probably tightly bound (cassette) and it seems unlikely that this interaction exceeds 1:1 stochiometry)

9c) from the occupied receptor, which processes can be amplified by elevated levels of cytosolic Ca^{2+} (make reference to your drawing) *(2 points)*
- at the level of adenylyl cyclase
- at the level of phopshorylase kinase

9d) Draw and explain how phosphorylase kinase activates glycogen-phosphorylase (make a comparison between the little and highly active state) *(6 points)*
- access of glycogen to catalytic cleft impeded by a loop of 9 amino-acids
- phosphorylation of serine-14 causes a conformational change that extends to the the catalytic site and displaces the loop of 9 amino-acids (tense to relaxed state)
- glycogen readily binds and is degraded into multi copies of glucose-1-phosphate (the enzyme acts both as a glucosidase and a phosphotransferase).

Name/first name	**grade/20**

Test-2

Text

Regulating the activity of protein kinases

The transfer of the third phosphate ($HPO4^{3-}$) of ATP on a protein is called phosphorylation. Phosphorylation is used in many instances to alter the activity of the proteins (case of enzymes) or to modify the interactions between cellular components (case of transcription factors, signaling complexes or intermediate filaments). The addition of one or more phosphate groups (pKa 6.7 and therefore dianionic around pH 7.2) changes in some cases the conformation of the protein and thus its activity. In others, phosphorylation creates a new interaction site (attachment site) or, conversely, prevents certain interactions. Phosphorylation is catalyzed by protein kinases. They are characterized by a catalytic domain of about 300 amino acids, consisting of two lobes, N-terminal and C-terminal, separated by a catalytic cleft. The two lobes are connected by a hinge. ATP is bound by the N-terminal lobe whereas the substrate binds the C-terminal lobe. The catalytic amino acid, an aspartate, is located at the C-terminal site of the cleft. The activity of protein kinases is tightly regulated by three mechanisms:

- Subcellular localization (to avoid or, conversely, facilitate access to their substrate)

- Phosphorylation of the activation segment

- Interaction with regulatory subunits

Regulation of protein kinase A (PKA) in four steps.

1) The newly synthesized protein is inactive (or incompetent), unable to phosphorylate substrate. Firstly, the activation segment is badly positioned and impedes access of substrate to the catalytic residue. Secondly, the αC-helix in the N-lobe, carrying a highly conserved glutamate, is turned towards the exterior and this prevents the right positioning of the γ-phosphate (third position) of ATP. As a result, the γ-phosphate is too far removed from both the catalytic residue (aspartate) and the receiving residue of the substrate; transfer a phosphate cannot occur.

2) Phosphorylation of the activation segment by an upstream activating protein kinase (in this case PDK1) causes an outward movement of the activation segment, liberating the substrate binding site and allowing access to the catalytic residue. It also causes an inward movement of the αC-helix and it pulls the N-lobe more tightly to the C-lobe. As a result, the glutamate participates in the (correct) positioning of the γ-phosphate of ATP. Everything is set for a phosphotransfer (compentent state of the kinase). It is important to realize that protein kinases act as a nanomachines; the transfer of phosphate only occurs if the γ-phosphate of ATP, the OH group of serine or threonine in the substrate and the catalytic amino-acid aspartate are situated within a radius of about 0.4 nm. A protein kinase is called "active" when these conditions are met! Activity is expressed by phosphorylation of substrate.

NB In the case of PKA, one of the substrates is an inhibitor, it is the regulatory subunit called RIα. It behaves as a substrate but lacks the serine or threonine and therefore cannot be phosphorylated ("pseudo-substrate"). It remains fixed and thus impedes access of other substrates. The second messenger cyclic adenosine monophosphate (cAMP), produced in reply to adrenaline for instance, binds RIa and causes its detachment. The kinase is active again.
3) Once the appropriate substrate binds, phosphate is transferred to its serine or threonine residue. Not all serines or threonines present in a protein are substrates; they have to be accessible and they need to be surrounded by a set of appropriate amino-acids (being imbedded in an appropriate phosphorylation motif).
4) Once the phosphorylation has occurred, both substrate and ADP leave the protein kinase. A new ATP and a new substrate take their place and another cycle ensues.

Deregulation of protein kinases (oncogenes) in cancer
In cancerous cells of different origins, numerous mutatations have been detected in genes that code for tyrosine protein kinases (these phosphorylate substrate on a tyrosine residue). These mutations generally facilitate the activation process, i.e. fewer post-translational modifications are needed to get them fully active. The lack of inhibitory control has significant consequences for the regulation of cell proliferation, migration and the ability of the cell to survive (avoid apoptosis). Examples of tyrosine protein kinase oncogenes are: Abl, Src, Yes, Lck, Syk and JAK.
End of text

Images of test 2 activation states of PKA (S5-S7 were used in preparation and S8 in the test)

Figure S5 realistic schematic representation

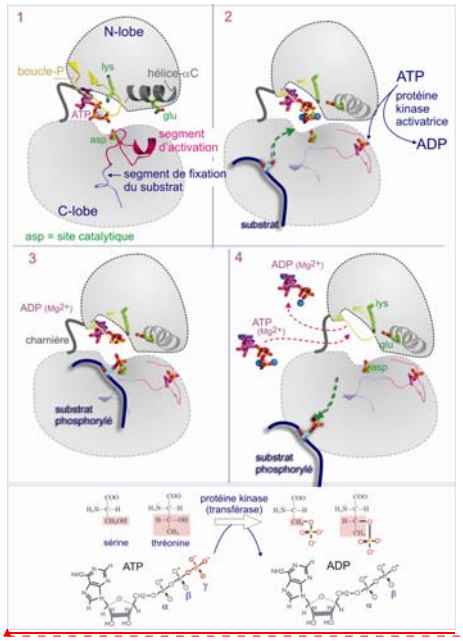


Figure S6 schematic representation

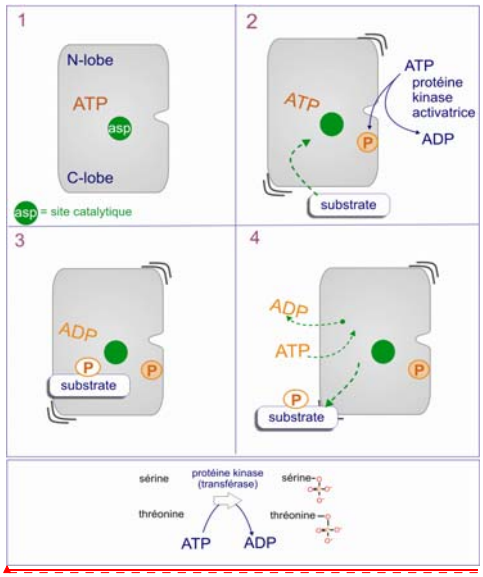


Figure S7 key words

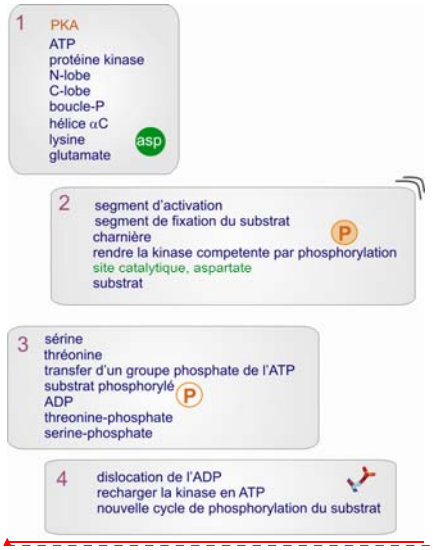
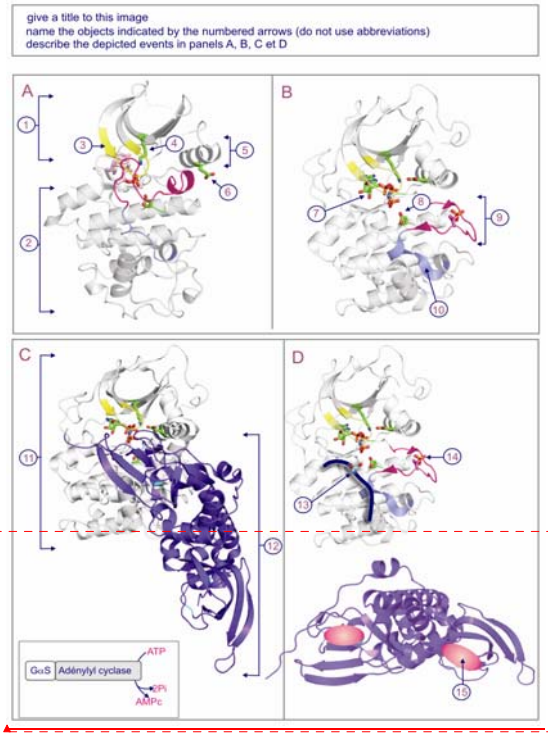


Figure S8 "raw" realistic representation



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Slide projected during the reading section of test 2

The following subjects are covered by the test:

- the identity and the orientation of the amino acids that play a role in the phosphorylation reaction
- the phosphorylation cycle
- the nature of the objects (protein, lipid, nucleotide, fatty acid)
- the processes that lead to activation of PKA
- you are asked to label an image of PKA

Question sheet accompanying image S8 of test 2

name/first name :	group :	Grade/20 :

Title *(2 points)*	Regulation of protein kinase activity (by phosphorylation and an inhibitory regulatory subunit)

Name the object and their function indicated by the number-labels *(1 point each)*	
1	N-lobe (binding of ATP)
2	C-lobe (binding of substrate)
3	P-loop (binding of ATP)
4	Lysine (precise coordination of ATP)
5	αC-Helix (positioning of glutamate)
6	Glutamate (precise coordination of ATP)
7	ATP (source of phosphate)
8	Aspartate, catalytic amino acid
9	Activation segment (needs to change position as it impedes access to the catalytic site)
10	Substrate binding segment
11	Catalytic subunit , protein kinase or protein kinase A
12	Regulatory subunit, inhibitor, RI-alpha or substrate
13	Substrate with serine (phosphorylation site)
14	Phospho-threonine residue of the activation segment
15	cAMP or second messenger

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Describe the event or object presented in the labeled pannels ((A), (B) etc *(4 points each)*	
A	Protein kinase (catalytic subunit) in its inactive (incompetent) state. It comprises two lobes, N- and C-lobe, separated by a catalytic cleft. The activation segment, in red, is badly positioned and impedes access to the catalytic residue. The αC-helix in the N-lobe, carrying a highly conserved glutamate, is turned towards the exterior and this prevents the right positioning of the γ-phosphate of ATP, which is too far removed both from the catalytic residue (aspartate) and the putative binding site of the substrate. The γ-phosphate cannot be transferred.
B	Phosphorylation of the activation segment by an upstream activating protein kinase (PDK1), outward movement of the activation segment, liberation of the substrate binding site, access to the catalytic residue, inward movement of the αC-helix, the N-lobed is pulled down a bit, correct positioning of the glutamate leading to a correct positioning of the β,γ-phosphates of ATP, everything is ready for phosphorylation ("compentent" state), the protein kinase is ready to bind and phosphorylate substrate
C	Binding of the RIalpha regulatory subunit (a pseudo substrate) to the substrate binding segment, the kinase is again inhibited.
D	Two cAMPs bind the RIalpha, leading to a conformational change (not shown in image) and subsequent detachment from the catalytic subunit. The protein kinase can now bind "real" substrates and transfer phosphate onto a serine or a threonine (phosphorylation reaction).

Encircle the correct answer				
16) cAMP is a : *(1 point)*	nucleotide	fatty acid	amino acid	sugar
17) the catalytic cleft is situated between objects: *(1 point)*	③ et ④	⑦ et ⑧	⑨ et ⑩	⑪ et ⑫
18) Object ⑫ is a : *(1 point)*	nucleotide	fatty acid	amino acid	sugar
19) Aspartate is a : *(1 point)*	sugar	tatty acid	nucleotide	amino acid

Fill in the squares corresponding to the correct (or incorrect) proposition (only one possibility per question)		
20) phosphorylation changes catalytic activity of enzymes because: *(1 point)*		a) binding of ATP is said to be an « activation » process.
		b) the phosphate carries energy (liberated in the hydrolysis of ATP) that brings the enzyme in an active state
		c) the phosphate changes the conformation of the enzyme and this causes a change in its activity
		d) GDP is converted into GTP in this changes the G-protein
21) In the context of signal transduction, the term « phosphorylation » by a protein kinase refers to the transfer of: *(1 point)*		a) a phosphate ($HPO4^{3-}$) from one protein to another
		b) a phosphate onto a GDP
		c) one or multiple phosphates, from GTP, onto a G-protein
		d) phosphates, derived from ATP, onto a polypeptide chain (protein)

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22) If the threonine in the activation segment would be replaced by a negatively charged amino acid (mutation), what could the possible consequence be for kinase activity? *(1 point)*		a) It could push the activation segment to the outside and render the kinase active without further need for phosphorylation
		b) It could keep the kinase in its inactive state because, just like phosphorylation, it would position the activation segment on top of the substrate binding site
		c) This mutation does not change anything as the activation segment does not play a role the regulation of kinase activity
		d) This mutation does not affect kinase activity because only phosphorylation of the substrate binding segment plays a regulatory role

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Test-3

Text the same as in test-1, images the same as S3 (year 1) and S1 (year 2) shown previously

Image S9 raw schematic representation of adrenaline-mediated activation of glycogen-phosphorylase

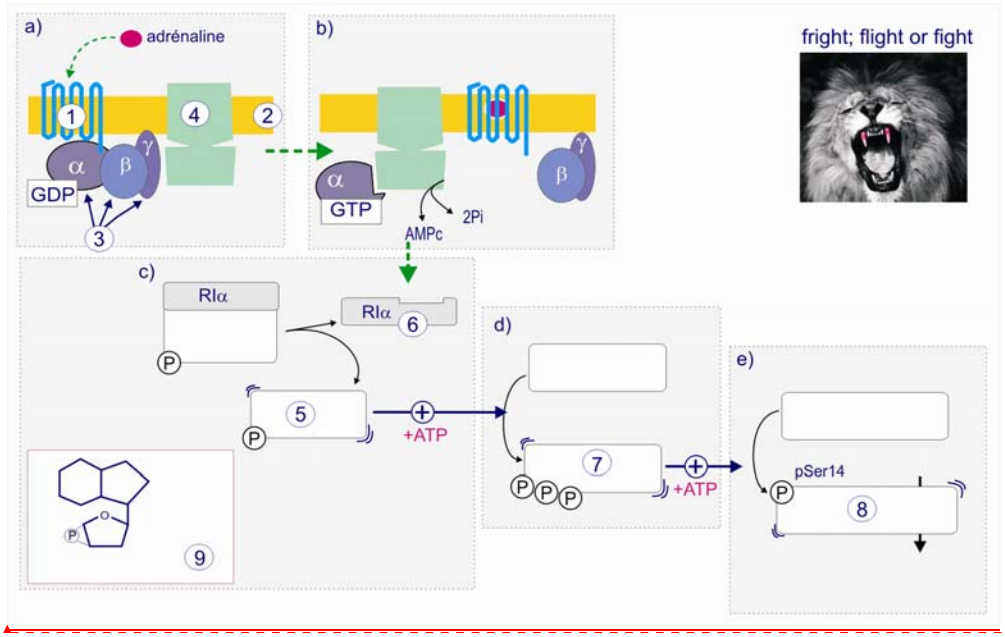

Image S10 raw realistic representation of adrenaline-mediated activation of glycogen-phosphorylase

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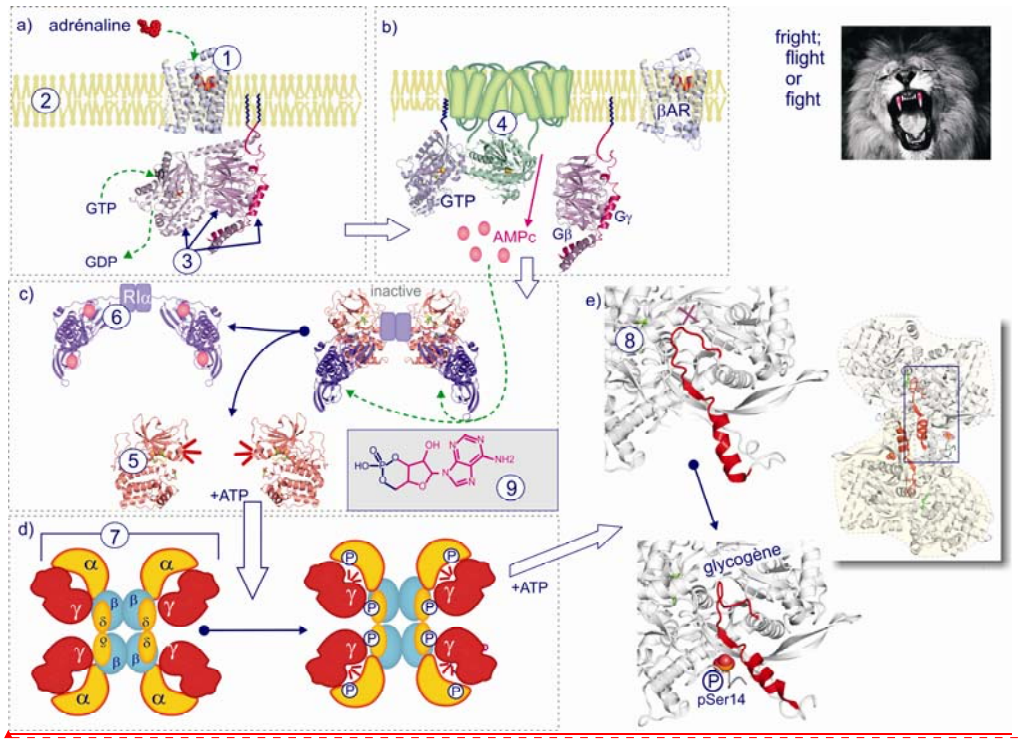

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Question sheet of test 3

Name / first name	grade/20
-provide a title for this image (in the space below) (10 points) Adrenaline-mediated activation of glycogen-phosphorylase (or equivalent)	
-provide a name (not an abbreviation) to the number-labeled objects :	
(1) adrenaline receptor (β-adrenergic receptor) (5 points)	
(2) plasma membrane (lipid bilayer) (5 points)	
(3) heterotrimeric GTP-binding protein (G-protein) (5 points)	
(4) adenylyl cyclase (enzyme responsable de la conversion d'ATP into AMPc + 2Pi (5 points)	
(5) protein kinase A (catalytic subunit, cAMP-dependent protein kinase) (5 points)	
(6) regulatory subunit of protein kinase A (inhibitor of protein kinase A) (5 points)	
(7) phosphorylase kinase (proteine kinase that phosphorylates glycogen phosphorylase) (5 points)	
(8) glycogen phosphorylase (5 points)	
(9) cyclic adenosine monophosphate (cAMP) (5 points)	

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-describe the events occurring in the panels indicated by the letter (a), (b) etc. Only use the space given below. It is advised to work on scrap paper first and chose the right wording before filling out the reply sheet.
a) binding of adrenaline to its receptor, activation of GTP-binding protein followed by dissociation of the alpha subunit (5 points)
b) dissociated G-protein complex, binding of the GTP-bound Gα-subunit to adenylyl cyclase leading to its activation and followed by the conversion of ATP into cAMP and 2Pi. The cAMP is a second messenger that diffuses into the cell. (5 points)
c) binding of cAMP to the regulatory subunit RIα, leading to its dissociation from the catalytic subunit (PKA) thereby liberating the catalytic site of the protein kinase (5 points)
d) phosphorylation and activation of phosphorylase-kinase (5 points)
e) phosphorylation (on serine-14) and activation of glycogen-phosphorylase, leading to the degradation of glycogen into multiple glucose-1-phosphates (ready to enter the glycolysis after conversion into glucose-6-phosphate).
Describe in more detail the mode of activation of the object shown in panel (e) (10 points)
The activated phosphorylase kinase (a serine/threonine protein kinase) phosphorylates the serine-14 residue of glycogen-phosphorylase. Due to its negative charge, this leads to a conformational change that extends to the catalytic site (binding site of glycogen) where a loop of nine-amino acids changes position (from a tense to a relaxed state). Glycogen now has better access and is degraded into multiple copies of glucose-1-phosphate. Through post-translation modification, initiated by adrenaline, the muscle cells are able to prepare an important production of glucose-6-phoshate in anticipation of intense labour.

Section II - Qualitative analysis of the realistic image ("unfolding" exercise)

The aim of this exercise is to let students unfold what they read in the realistic representation (figure S1). Fifteen volunteers were interviewed by tutors, one week after they had finished the tutorials, and their replies were written down (table S1). We have employed this exercise in order to obtain more detailed information of how students interpret the icons (symbols) in the image and how they combine this with verbal instructions (coherence formation) . The questions have no wrong or right answers and it is important that the interviewer does not push the students towards a unique (and "correct") interpretation.

Table S1 Questions and answers of the qualitative analysis of the realistic representation of the adrenaline to glycogen-phosphorylase pathway

In what order are you going to read this image, explain why	**prevalence**
-from left to right, in the order ABCDE, it is an alphabetic order, direction indicated by white arrows.	14
-it is difficult, very abstract, shapes not recognized, names recognized, for instance adrenaline, follow the arrows, that makes sense	1
What is represented by the yellow objects in A and B?	
Cell membrane characterized by a bilayer of lipids and embedded proteins	8
Cell membrane, lipid bilayer with heads (hydrophilic) on the outside and tails (hydrophobic) pointing to the inside	2
Lipid bilayer, you recognize the hydrophobic heads and the hydrophilic tails of fatty acids	1
A membrane because there are objects embedded in the bilayer	1
It is the molecule βAR because it is written βAR	1
The membrane of a cell	1
Cell membrane because there is a receptor embedded in the structure, adrenaline causes a reaction at the level of the receptor	1
Which objects represent proteins and why?	
Objects with colour, with subunits and sometimes anchored in the membrane, spiralled shapes as well, beta-barrels, phosphate bound objects	1
Round or twisted objects, with loops, tangled strands that suggest their 3D structure, they are generally brightly coloured	3
All objects except the membrane, nucleotides and adrenaline. You recognize them by their 3D structure	1
Heterotrimeric G-protein, protein kinases and glycogen phosphorylase are named proteins, they are represented by tangled strands	2
Proteins carry a phosphate, recognized by P	1
Ribbons represent proteins because the heterotrimeric G-protein is represented in a ribbon	1

fashion	
The helical shapes because these can be compatible with membranes (TM segments)	1
Structures with helices and beta-sheets are proteins, that is the way we have learned it (definition of protein), as well as objects with "protein names", a matter of habituation	2
Adrenaline is a hormone, not a protein, protein kinase A and glycogen phosphorylase are enzymes, but not sure if they are proteins. Receptors are proteins, so βAR is a protein and adenylyl cyclase is a protein.	1
All objects including adrenaline, a protein hormone. I recognize their form and size, habituation	1
Proteins have subunits and are generally big, not like GTP which is much smaller (nucleotide)	1
Which objects represent nucleotides and why?	
GTP, GDP, ATP, AMPc, things named by initials (for instance GTP signifies guanosine tri phosphate)	6
Pink structures	1
I do not distinguish nucleotides	2
Recognized by their structures as shown for cAMP (Lewis representation)	4
Bottom left, cAMP, is a nucleotide, you recognize the structure, I know that ATP, GTP and GDP are also nucleotides	1
Nucleotides make me think of DNA but I cannot indicate the objects in this image	1
Which objects are identical in section A and B and why?	
GTP, Gαs, membrane, Gβ & Gγ because the same representation, colour scheme and name, showing two different stages of the same process, just a change in orientation	9
The membrane, the tangled strands (G-protein), GTP	1
The membrane	1
Heterotrimeric protein, same representation	1
The helical elements are the same, the two sections show their displacement (dissociation) and interaction with the membrane	2
Plasma membrane, proteins linked to adhesion molecules, nucleotides	1
Which objects are identical in section C and why?	
RIalpha and their carrying red/orange subunits (PKA), they detach from each other with addition of cAMP, they have the same shape and colour, similar representations	12
The RIalpha's	3
How are the catalytic sites indicated in section C and D?	
By a trait, (three) red lines, showing activity	8
By a cleft in an otherwise round object	1
At the end of the green dotted line	1
By the presence of a P	1
Do not know	2
Where the substrate binds	2
What means "+ATP" in section D and E?	
Protein needs to bind ATP in order to be active, a change in conformation	1

ATP is added in order to trigger the reaction and arrive at the following step, the reaction requires ATP	2
ATP is added from outside the system	1
ATP is required for the binding (of phosphate), needed for phosphorylation, hydrolysis,	7
ATP is liberated from the two proteins (P) in section C and this is needed for the phosphorylation, ATP is also created in section E.	1
Interaction with an ATP molecule	1
Creation of ATP in going from one section to another (one section to another)	1
Transition from section C to D only occurs if there is sufficient ATP	1
What do the dotted green arrows represent?	
Illustrates an interaction protein-ligand that leads to a process, cascade of events	1
Illustrates movement to binding-site of nucleotides (cAMP or GTP or both)	9
They designate hydrolysis of GTP	1
Binding (of one element to another, for instance adrenaline to its receptor)	3
Catalytic sites to which cAMP binds	1
What does this sign (P) stand for?	
A phosphate group provided by ATP (or GTP)	2
(Addition of, fixing of) a phosphate (group)	11
phosphorus	2
Explain what you see in section E	
Glycogen phosphorylase before and after binding of phosphate, 3D structure, change in folding of the red loop, binding of glycogen (catalytic site accessible)	3
Glycogen phosphorylase, before and after binding of phosphate	3
A cluster of symbolic units where glycogen is going to fix	1
A cluster of symbolic units	1
Glycogen, of which the catalytic site contains a red protein, which changes position after phosphorylation, thus rendering the catalytic site accessible to glycogen	1
Binding system of glycogen phosphorylase inside a catalytic site	1
It concerns the chaining of an α-helix and a beta-sheet (parallel or antiparallel), these possess a catalytic site where glycogen binds	1
Folding changes around the catalytic site of glycogen, active and inactive states, mission to create an enzyme called glycogen phosphorylase	1
Binding of ATP accelerates the reaction that leads to binding of glycogen to the P-object, ATP-mediate activation of glycogen phosphorylase (glycogen converted into glucose-1-P)	2
Activation of glycogen phosphorylase	1
The addition of ATP closes the catalytic site because of binding of a phosphate group, removal of phosphate leads to activation; change of conformation.	
By combining the five sections; explain the process	
This image illustrates the different processes, starting with binding of adrenaline to its receptor (βAR), leading to a change in conformation (and activation) of glycogen phosphorylase (due to	4

ATP) (and finally giving rise to the production of ATP)	
Binding of adrenaline to its receptor, binding of Gαs (after dissociation of G-protein complex) to adenylyl cyclase, activation and production of cAMP, binding of cAMP to αRI, activation of PKA (binding of phosphate), activation of phosphorylase kinase, activation of glycogen phosphorylase.	7
Change of heterotrimeric G-protein, provision of adenylyl cyclase, conversion of ATP into cAMP and 2Pi, leading to the formation of PKA, leading to the production of ATP by phosphorylase kinase which finally is used for the phosphorylation of glycogen, leading to it s binding to the catalytic site.	1
This image represents the binding system of a glycogen phosphorylase inside a catalytic site	1
A cascade of events induced by adrenaline, leading to very rapid cascade of changes (one affecting the other) with the aim to allow, for instance, movement of an organism.	1
Binding of adrenaline induces separation of Gα and Gβ̃γ interacts with ATP and causes production cAMP, separation of PKA from RIα, PKA acts as ATP on phosphorylase kinase, which then acts as ATP to accelerate the binding of glycogen, and this leads to a new molecule charged with phosphate (P).	1
provide a title for the image	
A cascade of reactions following the binding of adrenaline and leading to the phosphorylation of glycogen phosphorylase	1
Scheme of cellular reactions to stress (which led to the release of adrenaline)	1
Protein cascade, from adenylyl cyclase to phosphorylation of glycogen	1
Mechanism of action of adrenaline	2
Signal transmission in the reaction of adrenaline to activation of glycogen phosphorylase	1
Different stages in the phosphorylation of glycogen phosphorylase	1
Formation of glycogen phosphorylase starting with adrenaline	1