

A MECHANISM FOR THE EVOLUTION OF PHOSPHORYLATION SITES

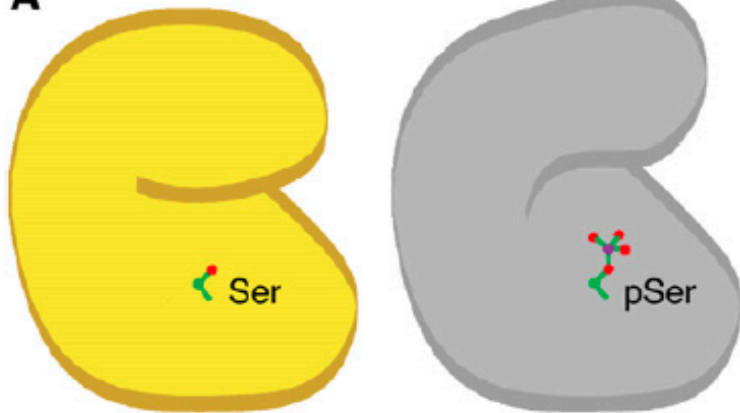
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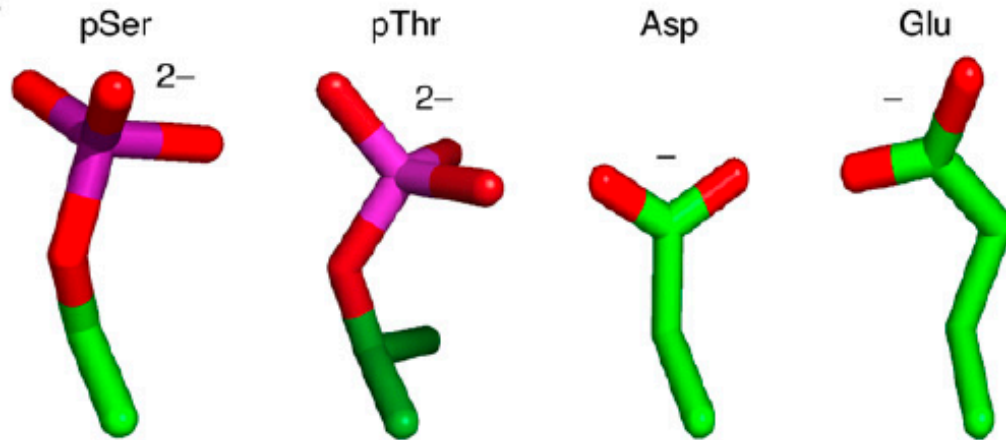
FEB 7, 2012

A

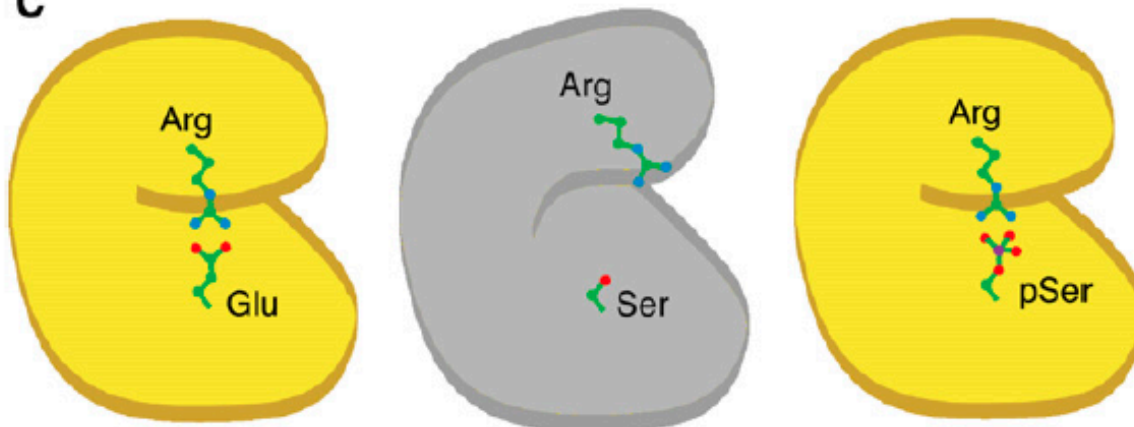


POSSIBLE MECHANISMS OF THE EVOLUTION OF PHOSPHORYLATION

B

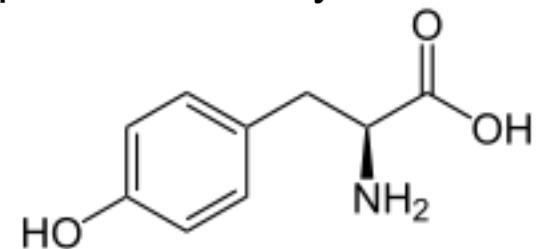


C



- A. Protein evolve first, then functionally important S/T/Y, then phosphorylation?
- B. Structures of pS, pT, D, and E
- C. pS and pT could restore salt bridges by acidic amino acids?

pY? Structurally dissimilar



CONCLUSION

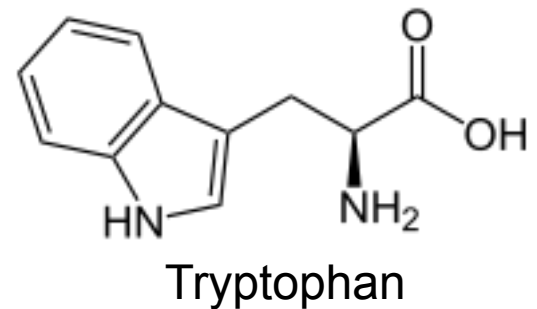
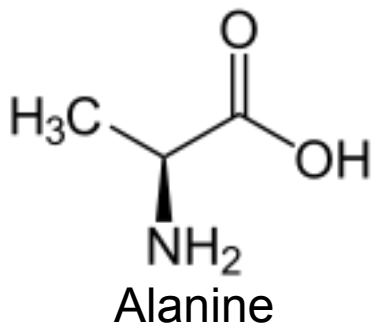
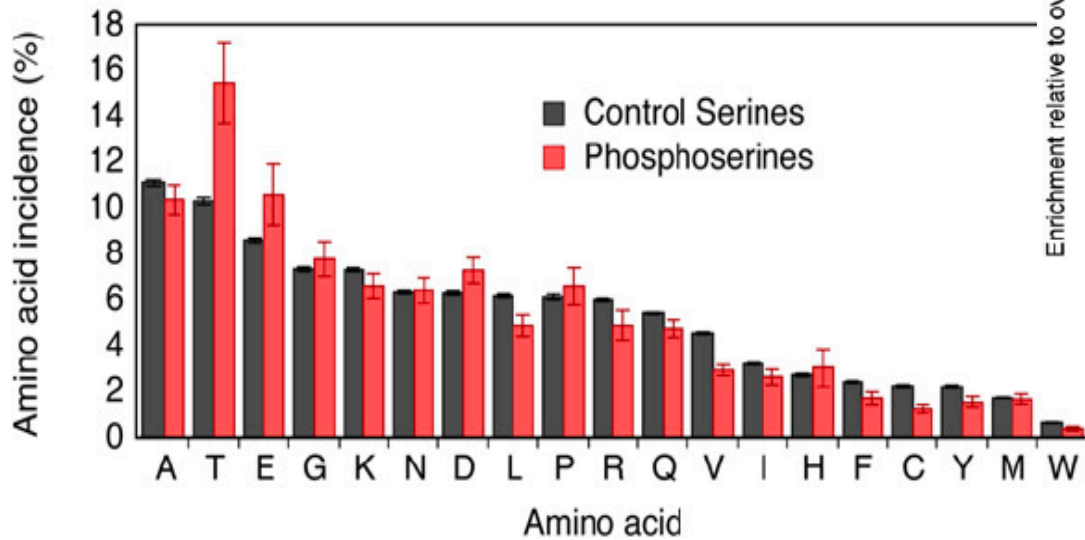
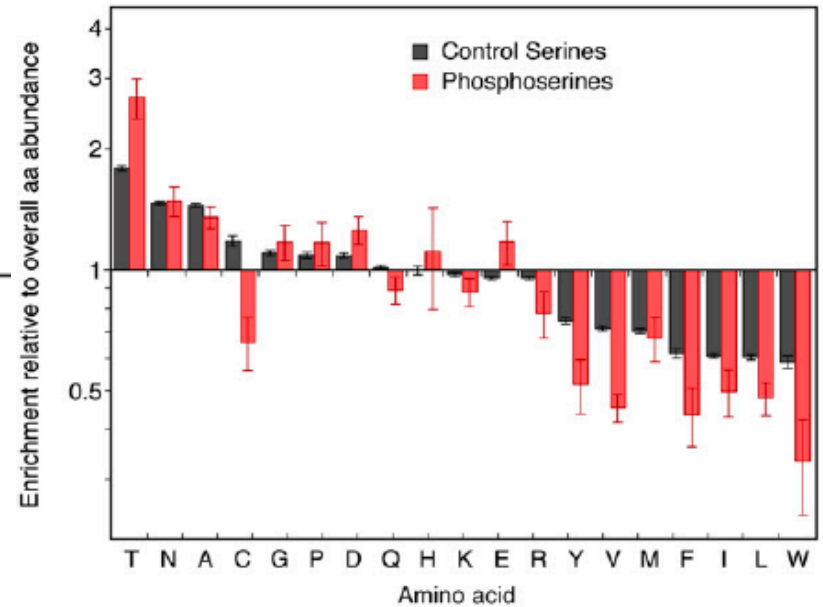
- ~5% of examined sites have evolved from acidic residues
- Such sites appeared both at the divergence of eukaryotes from prokaryotes and at subsequent times in evolution

D/E SUBSTITUTION RATES AT pS AND CONTROL SITES

A

	Control Ser	pSer	Control Ser
[<i>S.cerevisiae</i>]	...VSKVYAR	S	SVYDSRGN...
[<i>Acinetobacter</i>] 7.336E-104	...IVDIRARE	E	IILDSRGN...
[<i>Aeromonas</i>] 1.059E-102	...IVKVIGRE	E	IIDS RGN...
[<i>Aeropyrum</i>] 2.527E-96	...IERVWGL	Q	VLDS RGN...
[<i>Agrobacterium</i>] 5.617E-104	...ITDIIARE	E	IILDS RGN...
[<i>Alligator</i>] 2.838E-156	...ILKIHARE	E	IFDS RGN...
[<i>Alnus</i>] 1.568E-130	...ITHIKAR	Q	IFDS RGN...
[<i>Alternaria</i>] 4.500E-178	...ITKIHARS	S	SVYDS RGN...
[<i>Anas</i>] 4.099E-155	...ILKIHARE	E	IFDS RGN...
[<i>Aquifex</i>] 1.993E-109	...IKRVHGRE	V	VLDS RGN...
[<i>Arabidopsis</i>] 3.054E-134	...ITVVKAR	Q	IFDS RGN...
[<i>Archaeoglobus</i>] 3.812E-60	...IEDVHYR	V	VFDS RGN...
[<i>Aspergillus</i>] 8.487E-177	...ITKIHARS	S	SVYDS RGN...
⋮			
⋮			
⋮			

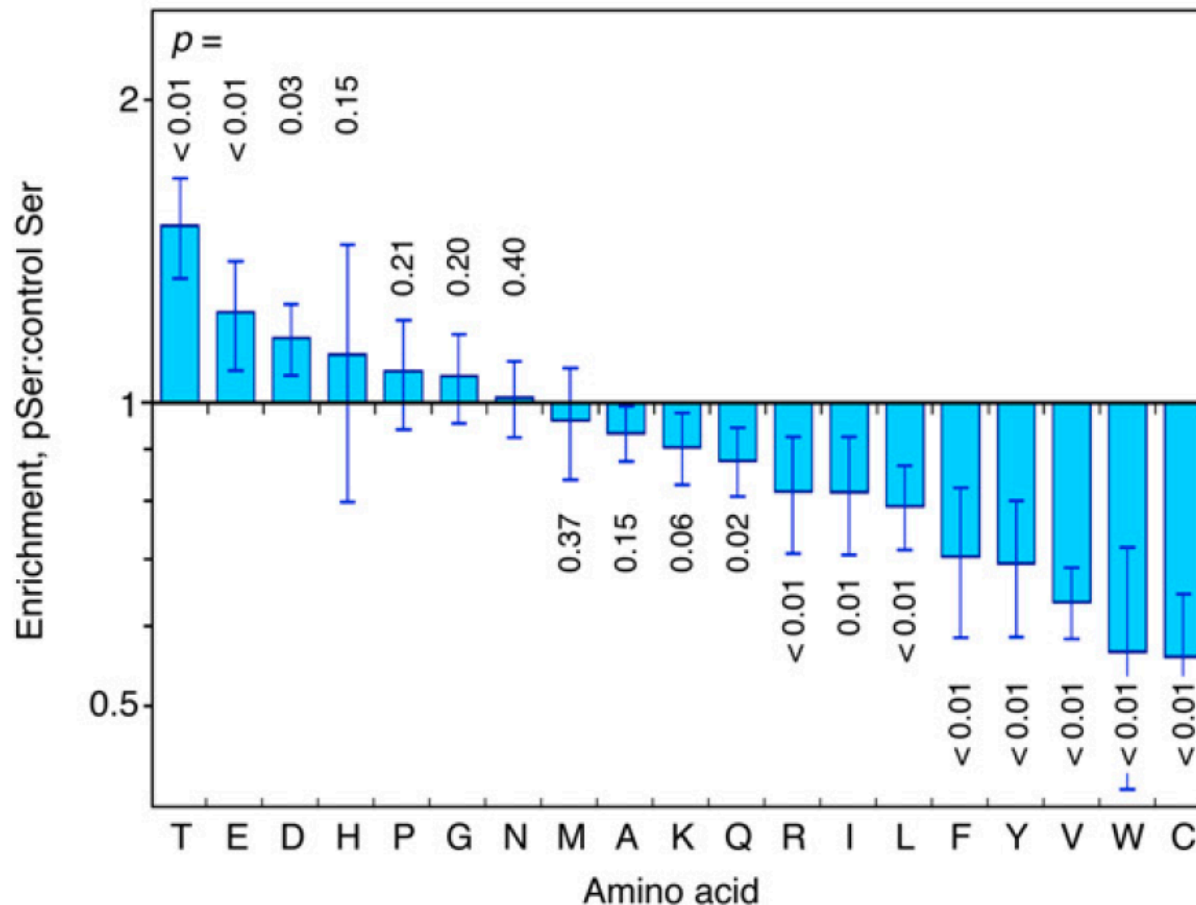
- Collect experimentally verified phosphorylation sites (7854pS, 1688pT, 566pY)
- Use Blast to find most closely related homologs
- Generate MSA using PROBCONS/ MUSCLE
- Calculate replacement ratios

B**C**

SUBSTITUTION RATES

T/E/D SIGNIFICANTLY ENRICHED AT pS SITES

D



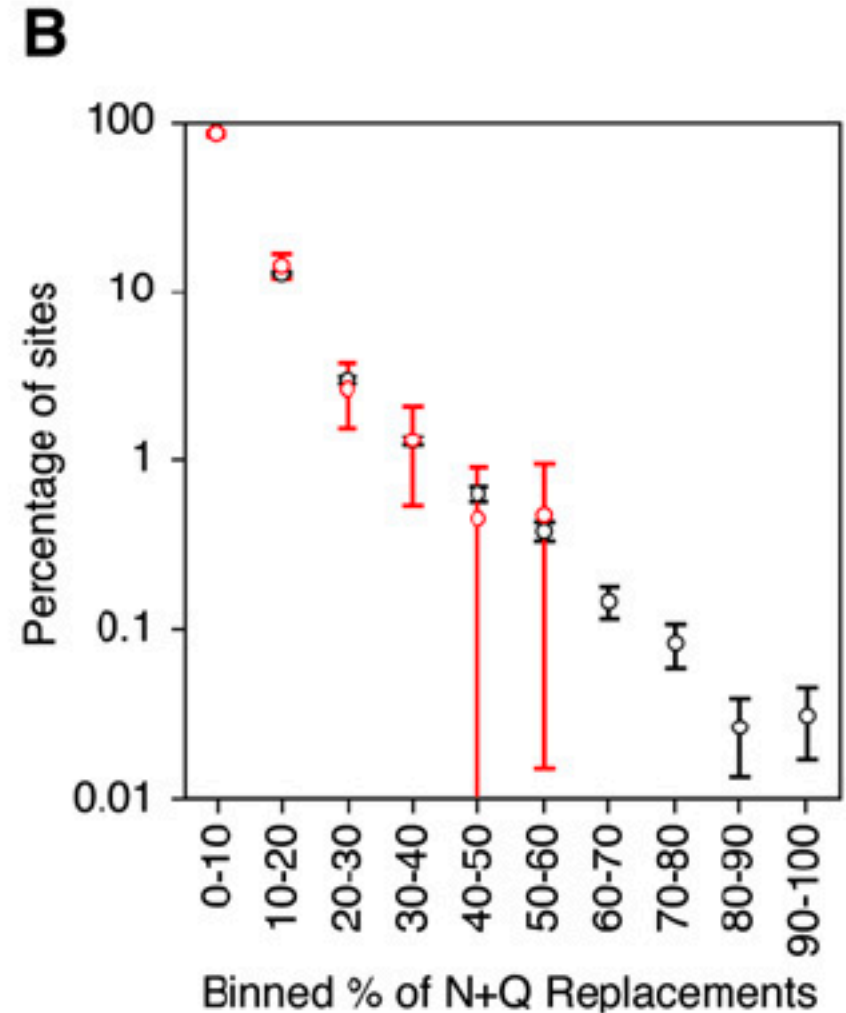
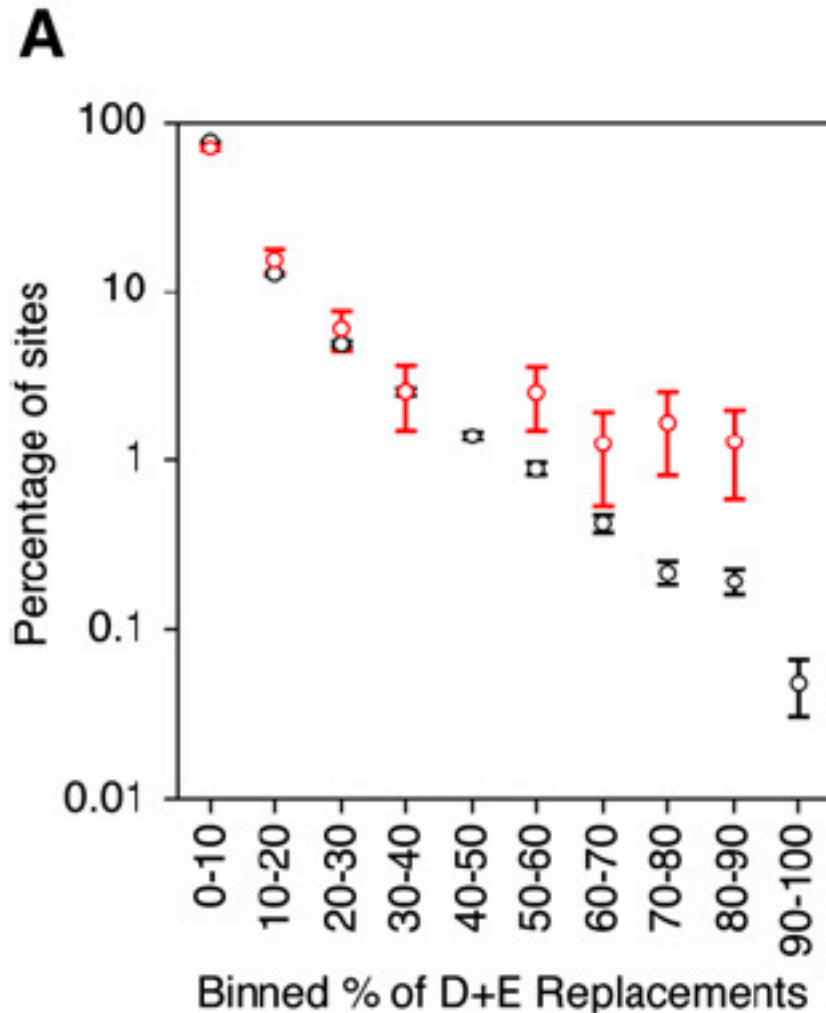
General enrichment because of surface preferences?

--- No, since Lys, Arg, Gln, and Asn are not enriched

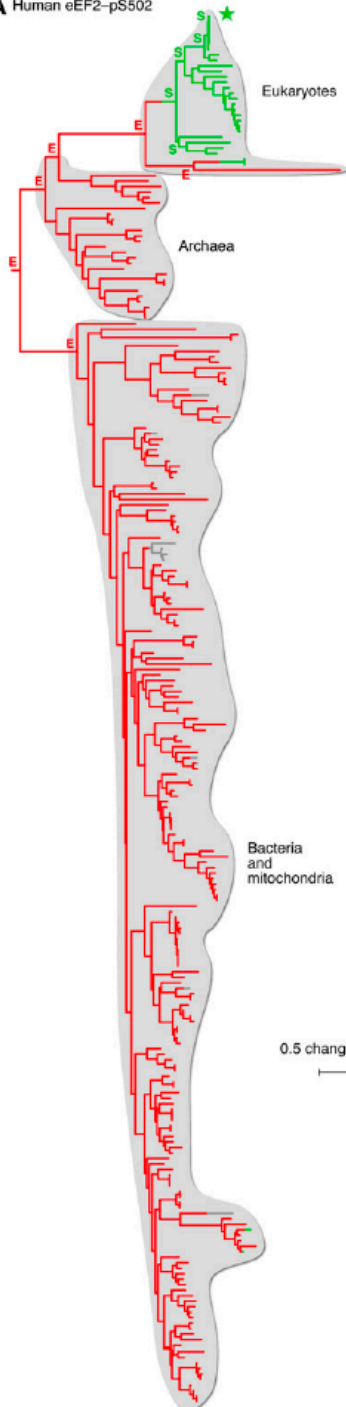
Error bars: bootstrapping

A SUBSET OF pS HAS VERY HIGH % OF D/E REPLACEMENT

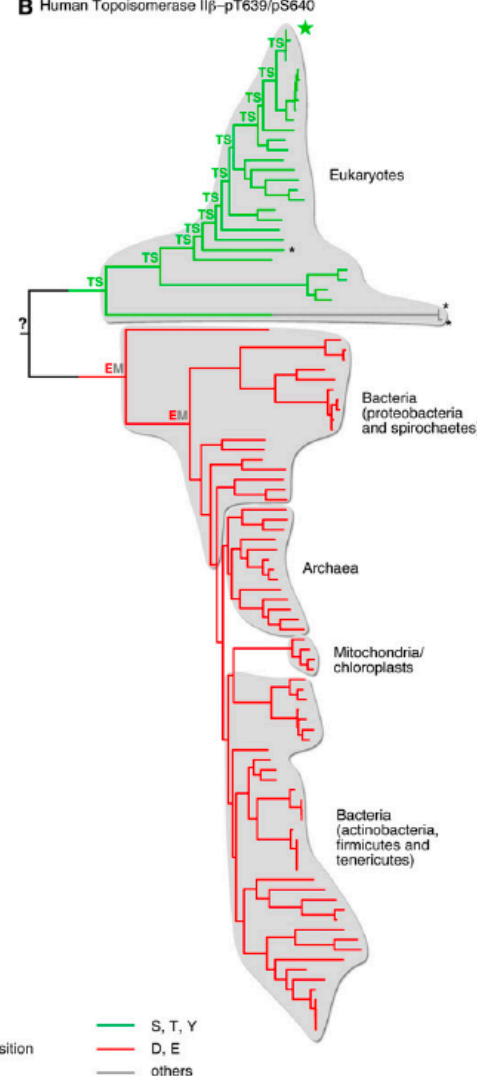
Structure similarity or charge similarity?



A Human eEF2-pS502



B Human Topoisomerase IIβ-pT639/pS640



C eEF2

	502
<i>Homo sapiens</i>	F S V S P V V R V A V
<i>Saccharomyces cerevisiae</i>	F S V S P V V Q V A V
<i>Escherichia coli</i>	F - P E P V I S I A V
<i>Synechococcus elongatus</i>	I - P E P V I S V A V
<i>Methanosarcina mazei</i>	H V S R P V V T V A V
<i>Sulfolobus acidocaldarius</i>	Y I S R P V V T I S V

D Topo II

	639 640
<i>Homo sapiens</i>	Y K G L G T S T A K E
<i>Saccharomyces cerevisiae</i>	Y K G L G T S L A Q E
<i>A. polyphaga mimivirus</i>	Y K G L G T S D P A E
<i>Escherichia coli</i>	F K G L G E M N P S Q
<i>Mycoplasma genitalium</i>	Y K G L G E M N A D Q
<i>Archaeoglobus profundus</i>	F K G L G E M N P E Q
<i>Methanosarcina mazei</i>	Y K G L G E M N P E Q

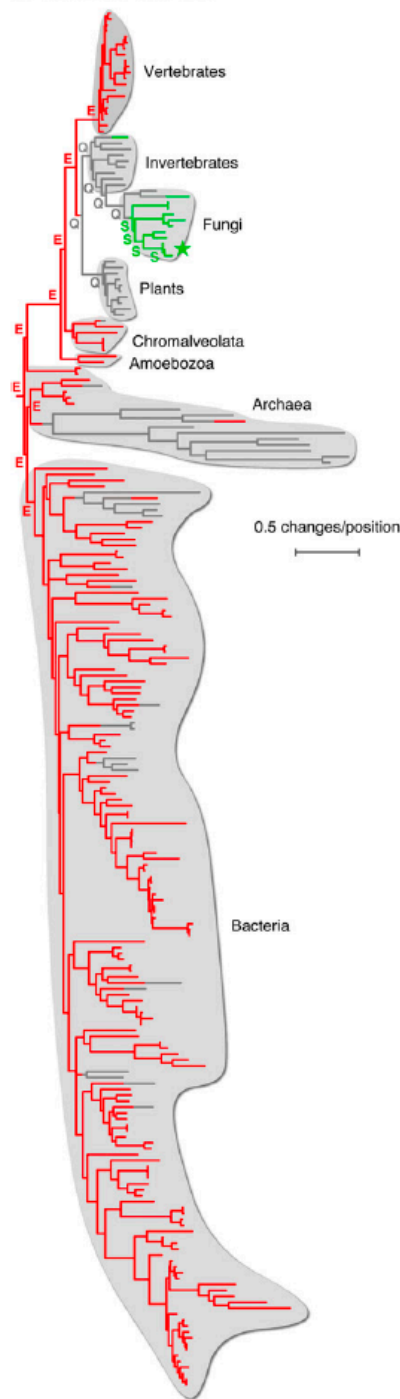
EMERGENCE OF PHOSPHORYLATION SITES AT THE PROKARYOTIC/EUKARYOTIC SPLIT

PhyML for trees
FASTML to infer internal nodes

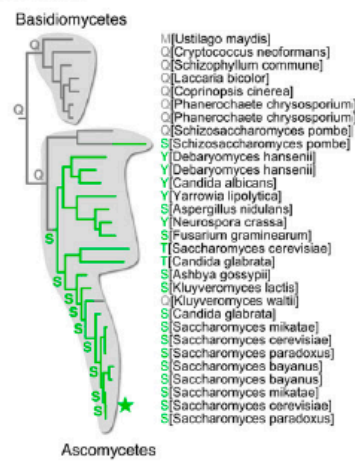
eEF2 : a translation factor required for the translocation step in protein synthesis

DNA Topoisomerase II: a conserved enzyme that catalyzes the passage of one DNA duplex through another.

A *S. cerevisiae* Eno1-pS10



B Fungal enolases



— S, T, Y
— D, E
— others

C Enolase

	10									
<i>Homo sapiens</i>	W	A	R	E	I	L	D	S	R	G
<i>Drosophila melanogaster</i>	K	A	R	Q	I	Y	D	S	R	G
<i>Saccharomyces cerevisiae</i>	Y	A	R	G	V	Y	D	S	R	G
<i>Arabidopsis thaliana</i>	K	A	R	Q	I	F	D	S	R	G
<i>Methanosarcina mazei</i>	H	A	R	E	I	L	D	S	R	G
<i>Sulfolobus acidocaldarius</i>	K	G	Y	E	I	I	D	S	R	A
<i>Escherichia coli</i>	I	G	R	E	I	I	D	S	R	G
<i>Synechococcus elongatus</i>	T	A	R	E	I	L	D	S	R	G

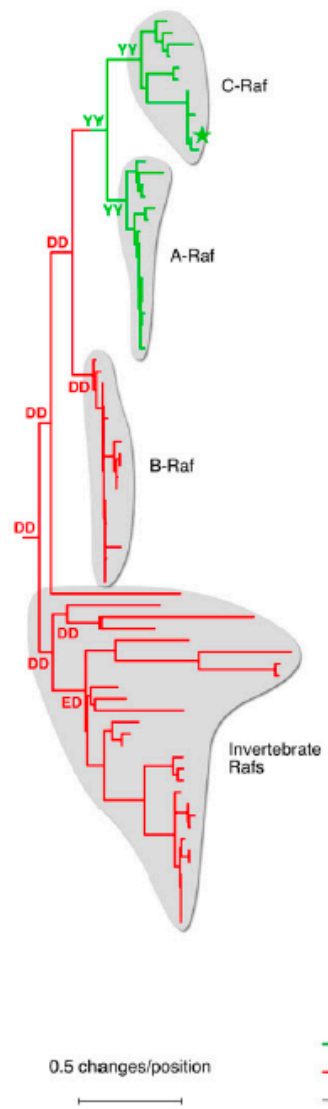
EMERGENCE OF PHOSPHORYLATION SITES LATER IN EVOLUTION

Glu → Gln → S/Y ?

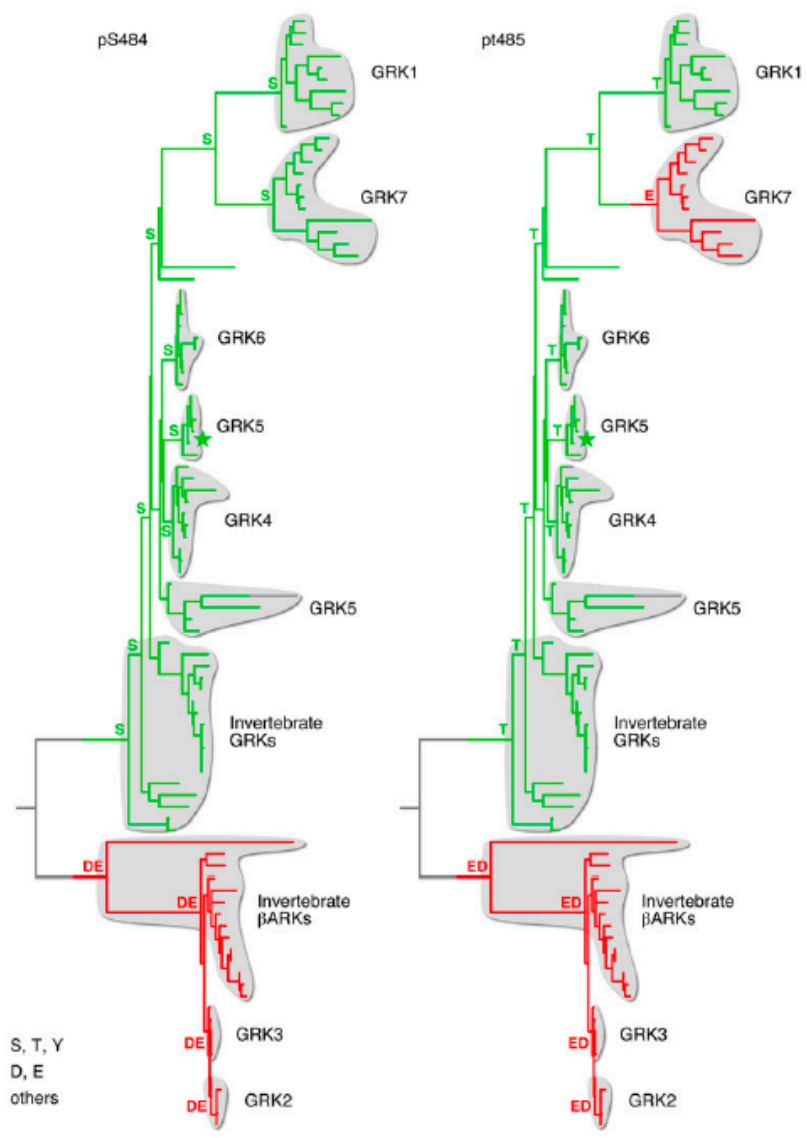
Gln can act as intermediaries in the evolution of phosphosites

Enolase: a key enzyme in glycolysis and glyconeogenesis.

A Human C-Raf-pY340 and pY341



C Bovine GRK5-pS484 and pT485



EMERGENCE OF PHOSPHOSITES IN PARTICULAR PARALOGS OF A MULTIPARALOG PROTEIN FAMILY

Raf: kinase
GRK: G-protein-coupled receptor kinases

Phosphosites to acidic residues?

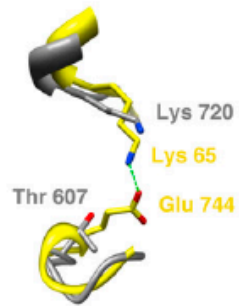
B Raf

	340	341
Human A-Raf	D S G Y Y W E V P P S E V	
Human C-Raf	D S S Y Y W E I E A S E V	
Human B-Raf	D S S D D W E I P D G Q I	
<i>Ciona intestinalis</i> Raf	D S N E D W E V P E E E I	
<i>Drosophila melanogaster</i> Raf	S S E E M W N I L A E E I	
<i>Apis mellifera</i> Raf	E S I E D W E I P A D E I	
<i>Hydra magnipapillata</i> Raf	D S N D D W E I K E G E I	

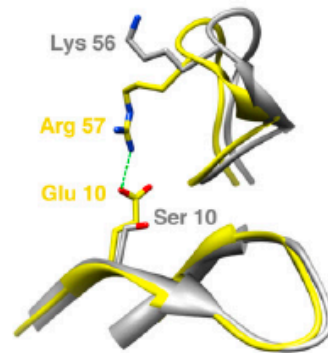
D GRK

	484	485
Bovine GRK1	G A F S - T V K G V	
Bovine GRK2 (betaARK1)	G S F D E E D T K G I	
Bovine GRK3 (betaARK2)	G S F D E E D T K G I	
Bovine GRK4	E Q F S - T V R G I	
Bovine GRK5	E Q F S - T V K G V	
Bovine GRK6	E Q F S - T V K G V	
Bovine GRK7	D D F S - T V R G I	
<i>Drosophila melanogaster</i> GPRK1	G S F D E E D T K G I	
<i>Drosophila melanogaster</i> GPRK2	E Q F S - T V K G V	

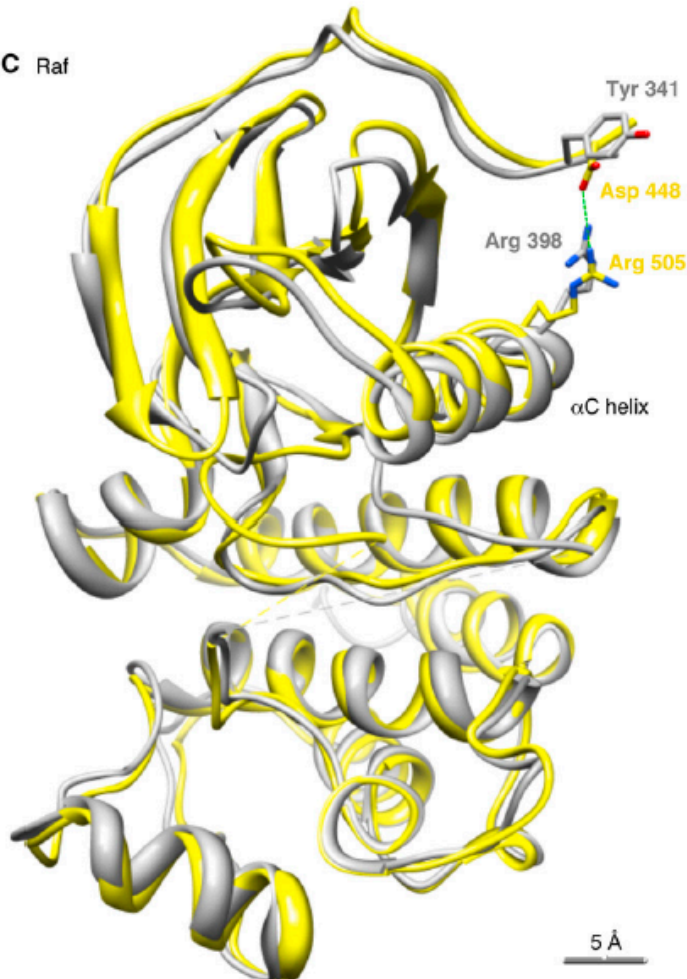
A Topo II



B Enolase



C Raf



STRUCTURES OF TOPO II, ENOLASE, AND RAF

- A. Topo II in *S. cerevisiae* and in *E. coli* (yellow)
3.1Å V.S. 7.3 Å
- B. Eno 1 in *S. cerevisiae* and in *E. coli* (yellow)
2.7Å V.S. 12.8 Å
- C. Human B-Raf (yellow) and C-Raf
3.5Å V.S. 7.7 Å

CONCLUSION

- **Acidic residues D/E were found to be present at the positions of phosphosites more often than they were at control serines.**
- **Other charged and polar amino acids did not, indicating enrichment is not simply because of surface preference.**
- **No similar enrichment for N/Q, indicating the importance of negative charge in this enrichment.**
- **~5% of examined sites (234, with high confident set of MSA, those with at least 60 homologs) have evolved from acidic residues**
- **Such sites appeared both at the divergence of eukaryotes from prokaryotes and at subsequent times in evolution**

DISCUSSION

- **This study provides an explanation for the fact that phosphorylation sometimes activates proteins;**
- **Both D/E to S/T/Y and S/T/Y to D/E are observed;**
- **Those phosphosites located are at well conserved and structured regions; --- allosteric regulation instead of bulk electrostatic effects or the generation of phosphoepitopes**