

# THE POTENTIAL RELATIONSHIP OF KINASE SUPPRESSOR OF RAS AND THE GLUCOSE PATHWAY

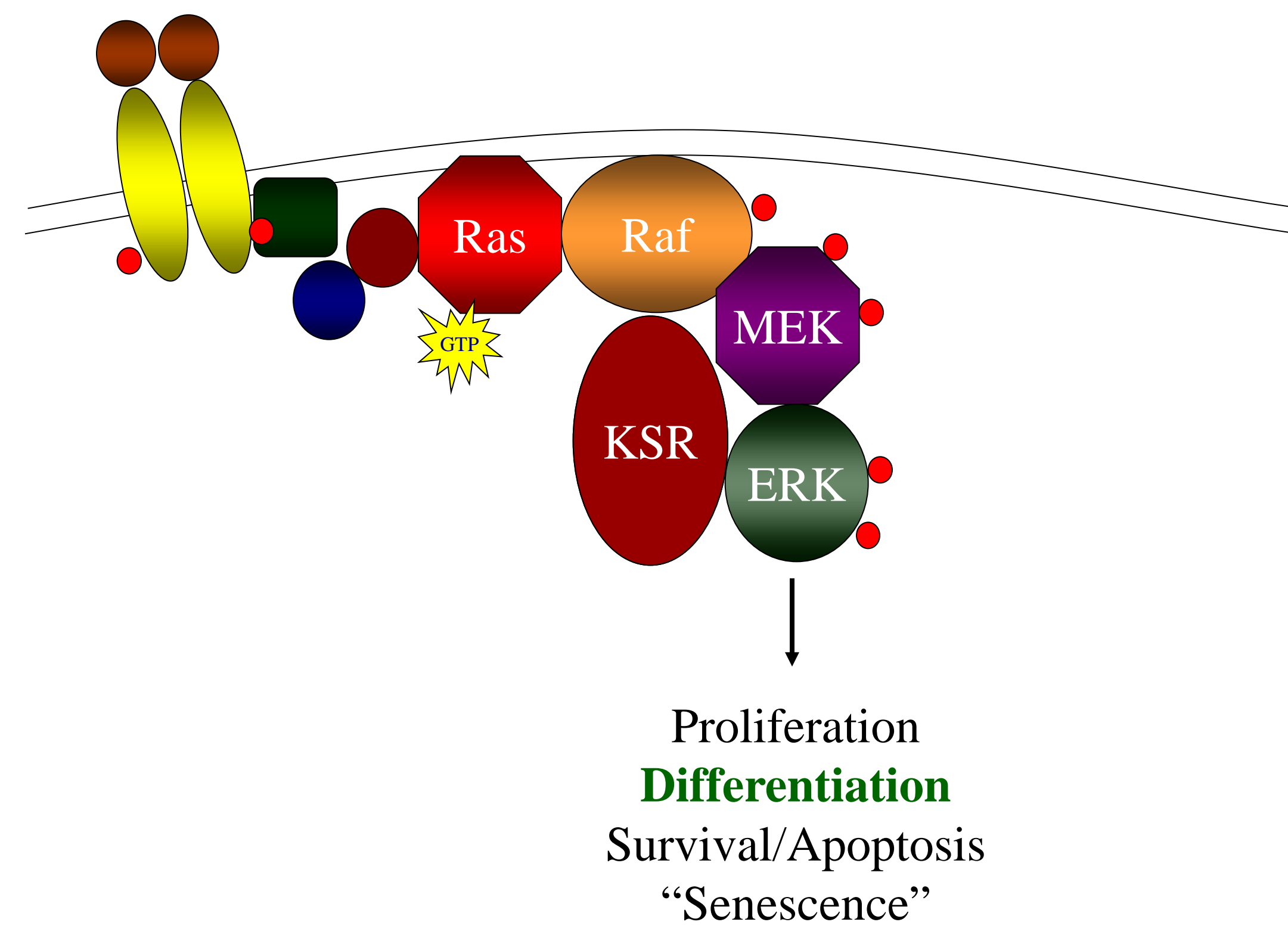
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Kinase Suppressor of Ras (KSR) is a molecular scaffold protein and positive effector of the Raf/MEK/ERK kinase cascade of the Ras pathway. The Raf/MEK/ERK pathway signals the cell to survive, undergo apoptosis, differentiate, or proliferate. KSR has been shown to directly regulate intensity and duration of ERK activation, which regulates the proliferative potential of a cell. Oncogenic potential of Ras is also regulated by KSR. What isn't known about KSR is how it affects glucose uptake. When cells are put under stress, such as glucose starvation, many triggers are activated. AMPK kinases are activated when there is an increase in AMP: ATP ratio. One of these AMPK kinases is CTAK-1. CTAK-1 is bound to KSR, possibly indicating a direct relationship to the glucose pathway and KSR activation. We hypothesize that when there is a glucose starvation more KSR will be expressed to counteract the lack of glucose, showing that KSR has a role in glucose uptake. Starving KSR 1.1 and clones of KSR1.1 (Mouse Embryonic Fibroblasts) for glucose over varied amounts of time will help determine the relationship of KSR and the glucose pathway. A correlation between glucose starvation and KSR production was seen in MEF's. *This research was funded by the Nebraska INBRE Grant Number: # P20 RR16469-05.*

## Introduction

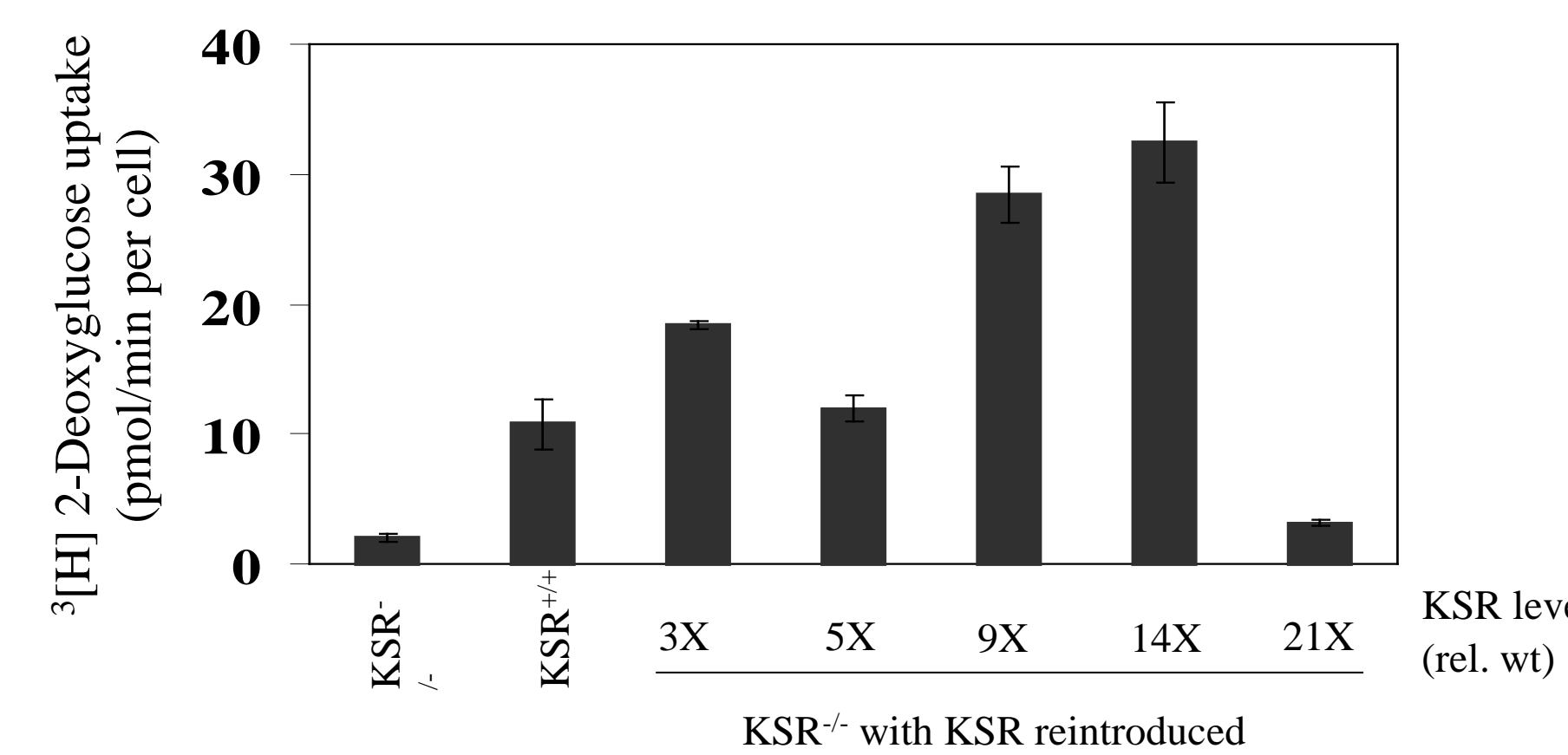
The Raf/MEK/ERK pathway, also known as the MAPK pathway, is a kinase cascade that signals the cell to survive, proliferate, differentiate, or undergo apoptosis. Kinase Suppressor of Ras (KSR) is a molecular scaffold and positive effector of the Raf/MEK/ERK kinase cascade shown in figure below. KSR has been shown to directly regulate intensity and duration of ERK activation, which regulates the proliferative potential of a cell. Oncogenic potential of Ras is also regulated by KSR.

**Figure 1**  
KSR1 is a molecular scaffold for the Raf/MEK/ERK kinase cascade



Another potential role of KSR is as a regulator of glucose uptake. When cells are put under stress, such as glucose starvation, many triggers are activated. AMPK kinases are activated when there is an increase in AMP: ATP ratio. One of these AMPK kinases is CTAK-1. CTAK-1 is bound to KSR, possibly indicating a direct relationship to the glucose pathway and KSR activation. Previous experiments in the Lewis lab show a positive relationship between KSR and glucose uptake shown in Fig. 2.

Starving KSR 1.1 cell lines for glucose will further show a positive relationship between KSR and glucose. We hypothesize that when glucose levels are low, more KSR will be expressed to counteract the stress of low glucose. If KSR1.1 MEF's are starved for longer periods of time more KSR will be expressed. We will then look at the glucose starvation in clones of KSR1.1. These clones express KSR at varied levels.



**Figure 2**  
Glucose levels were measured in KSR<sup>-/-</sup>; KSR<sup>+/-</sup>; KSR<sup>-/-</sup> with 3X, 5X, 9X, 14X, and 21X KSR reintroduced. 14X showed the highest uptake of glucose. The low glucose uptakes shown in the 21X KSR reintroduced agrees with previous evidence that KSR is more effective at low to medium expression levels, rather than high expression levels

## Materials and Methods

Cell Line	Characteristics	Special Conditions
KSR 1.1	KSR <sup>-/-</sup> MEF with KSR reintroduced	Sorted by Flow Cytometry
Clone 15	KSR1.1 Clone, expresses KSR .8X the wildtype	Sensitive Growing Conditions
Clone 27	KSR 1.1 Clone, expresses KSR 1X the wildtype	Sensitive Growing Conditions
Clone 15	KSR 1.1 Clone, expresses KSR 3X the wildtype	Sensitive Growing Conditions

\*Mouse Embryonic Fibroblasts (MEF's) cell lines used are shown in the above figure

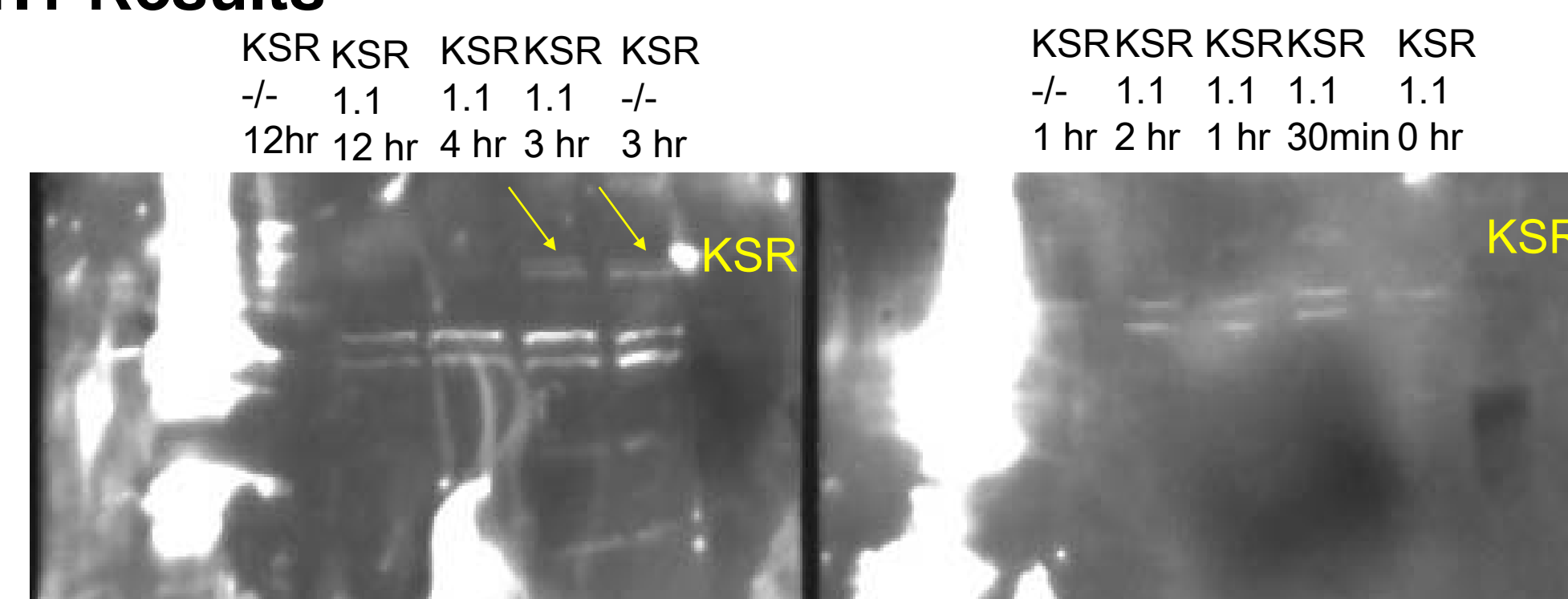
\*KSR 1.1 and KSR<sup>-/-</sup> (control) cell lines were incubated in no glucose media for 0 hr, 0.5 hr, 1 hr, 2 hr, 3 hr, 4 hr, and 12 hrs and then lysed open with CellLytic-M mammalian cell lysis reagent (SIGMA)

\*Clones were incubated in no glucose media for 0 hr, 2 hr, 3 hr and then lysed open with CellLytic-Mammalian cell lysis reagent (SIGMA)

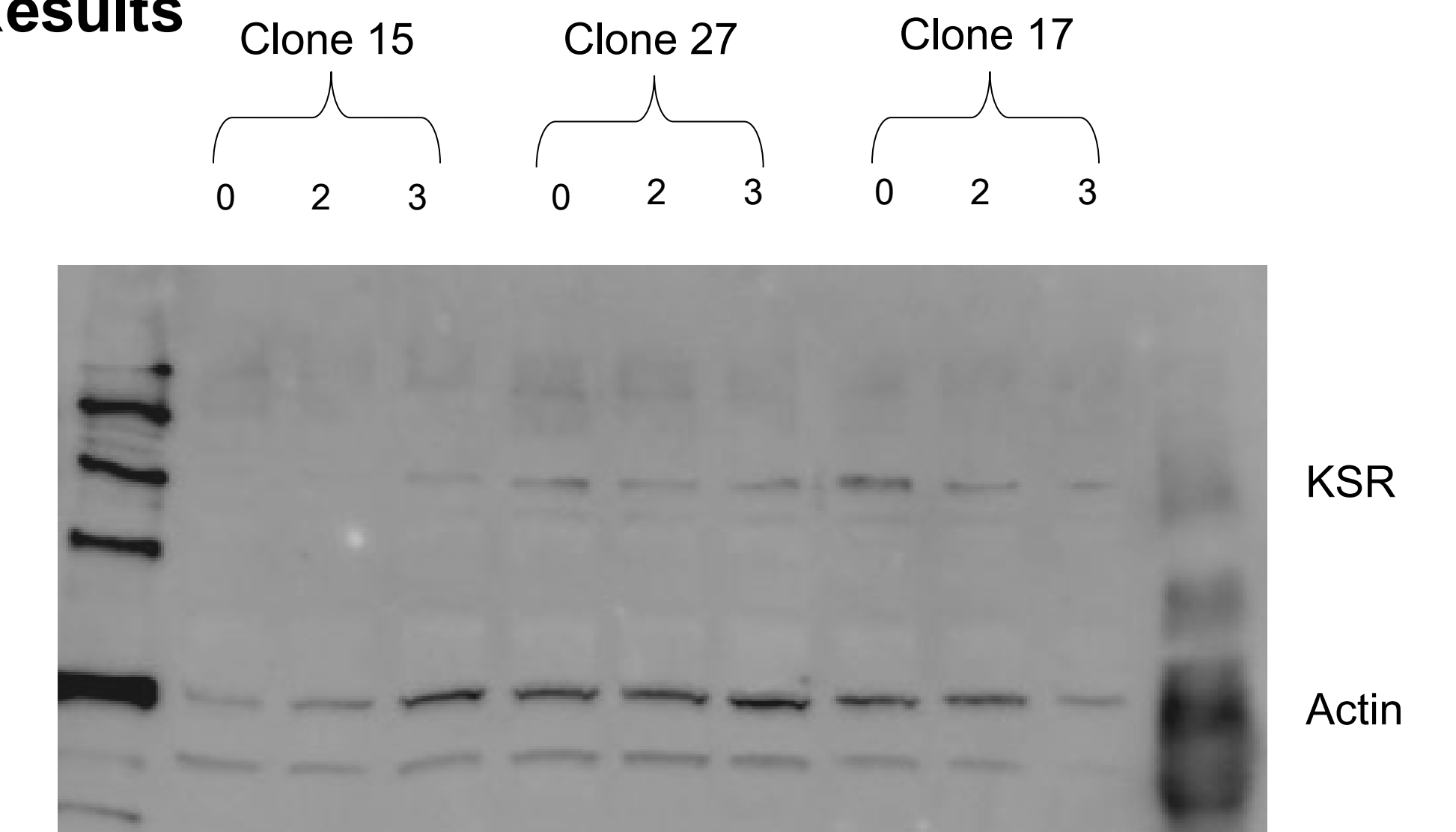
\*Lysates were utilized in a Western Blot utilizing KSR (M.W.:124 kDa) rabbit antibody, ERK (M.W.:44 kDa) rabbit antibody, and Actin (M.W.:43 kDa) rabbit antibody (Santa Cruz Biotechnology) and visualized via goat anti-rabbit secondary conjugated to HRP (Zymed Labs).

\*Chemiluminescence reagents were used to identify banding, and visualized using a UVP BioChemi gel documentation system.

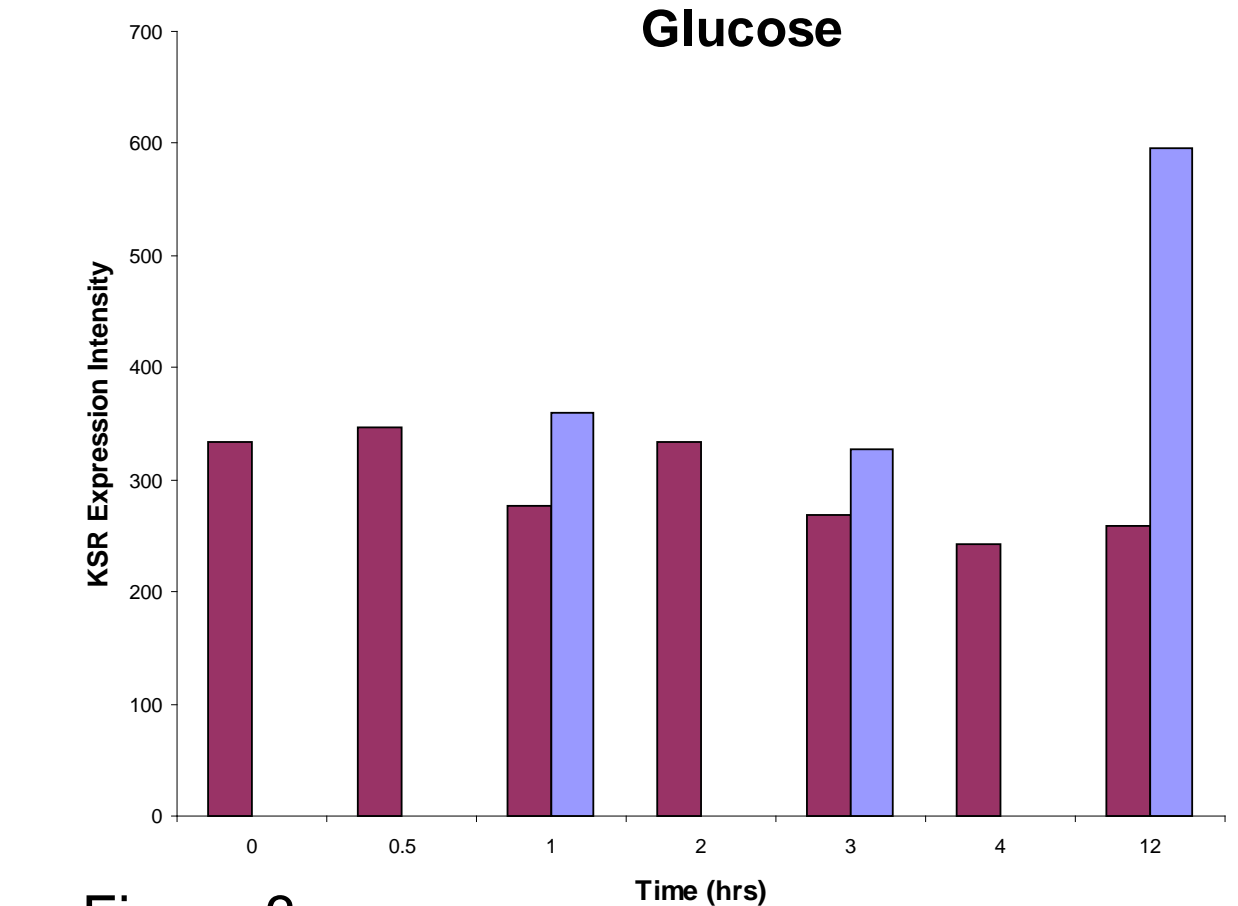
## KSR 1.1 Results



## Clone Results

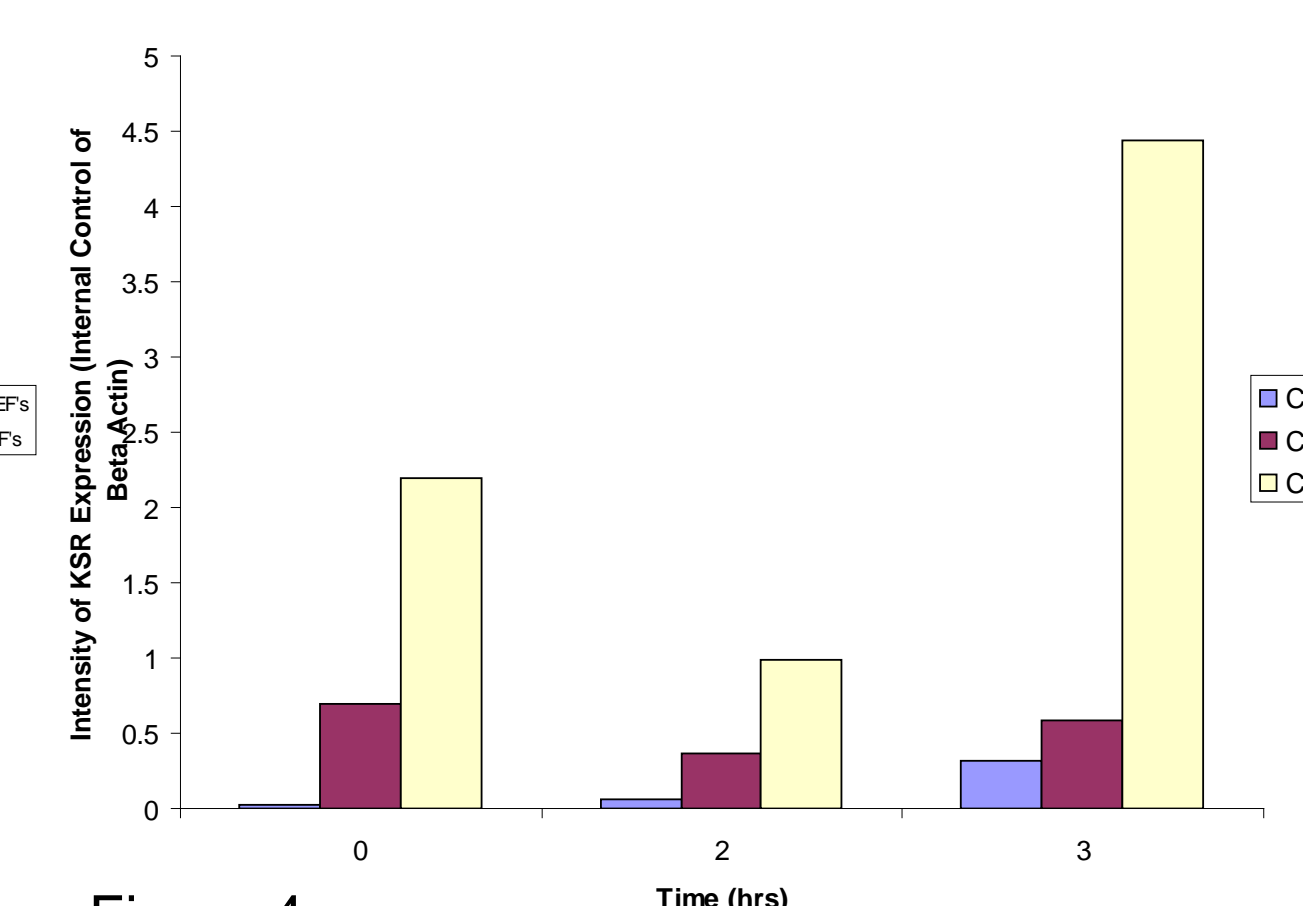


## KSR1.1 and KSR<sup>-/-</sup> MEF's Starvation of Glucose



**Figure 3**  
The intensity of KSR expression was measured using UVP BioChemi gel documentation system. KSR expression in KSR 1.1 increases when there is a starvation for glucose for 2hrs, and then decreases when there is prolonged glucose starvation.

## KSR 1.1 Clones Starved for Glucose



**Figure 4**  
The intensity of KSR expression was measured using UVP BioChemi gel documentation system. KSR expression in the Clone 15 cell lines increases when there is a glucose starvation for 3hrs. There is a decrease in KSR expression in Clone 17 and Clone 27 cell lines and then an increase when the cell are starved for glucose for 3hrs.

## Discussion and Conclusion:

What was hypothesized is that KSR expression will increase when there is a glucose starvation. KSR 1.1 results show that KSR was expressed during prolonged glucose absence, peaking at 2hrs, but there is a slight decrease of KSR expression at 1 hr. However, both KSR 1.1 and KSR<sup>-/-</sup> cell expressed KSR, showing a contamination of the KSR<sup>-/-</sup>.

All three clone lines show a change in KSR expression, when the clones are starved for glucose. Clone 15 has a steady increase of KSR expression, while Clone 27 and Clone 17 decrease in KSR expression at 2 hrs, and then increase again at 3 hrs.

The slight decrease in KSR expression before it reaches a peak is not yet explainable. By repeating the experiment with multiple samples of each lysate, will help to explain these results. In conclusion, there is a link between levels of KSR being expressed and glucose starvation, however further data is needed to demonstrate cause/effect of KSR-glucose relationship.